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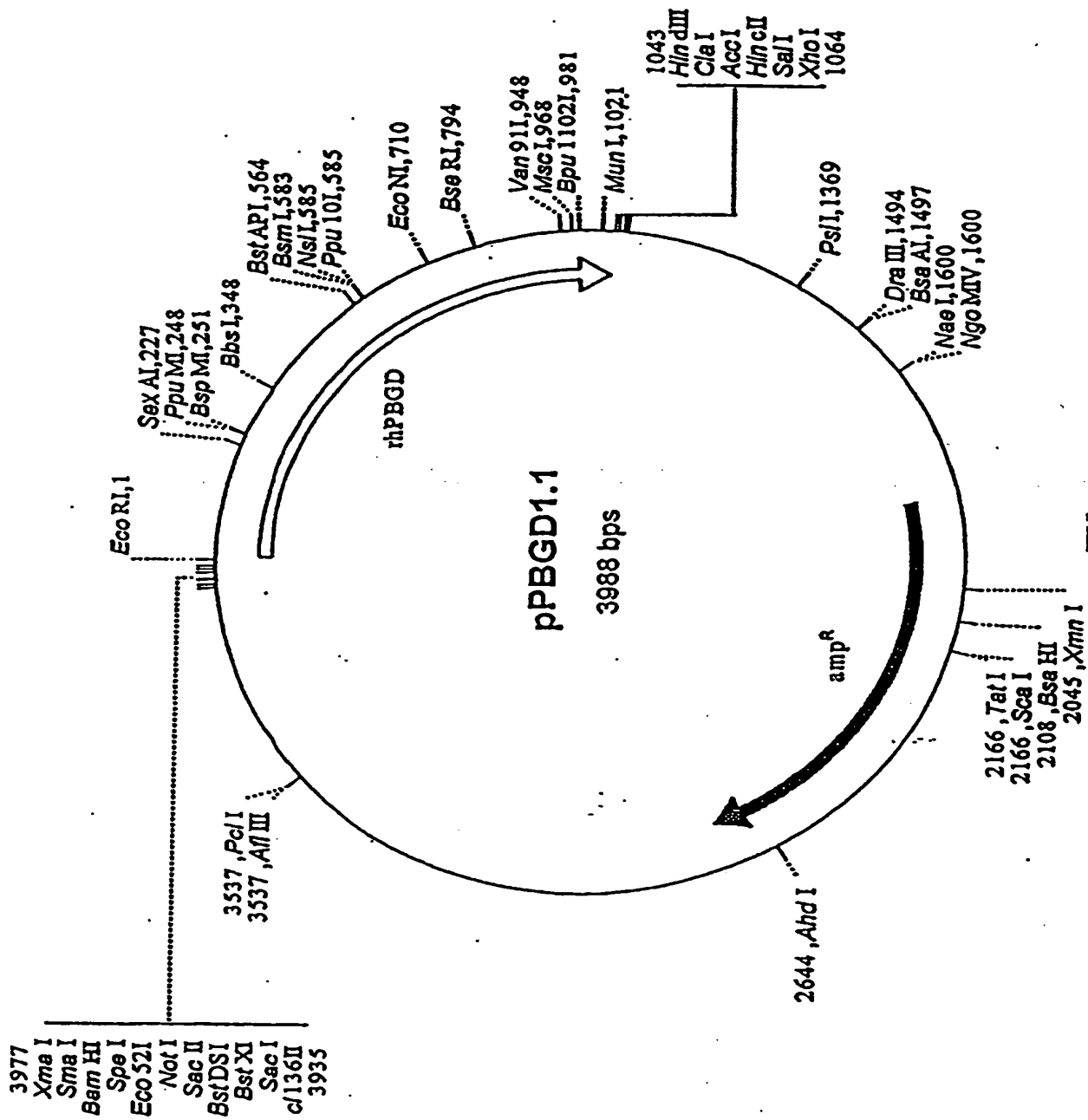
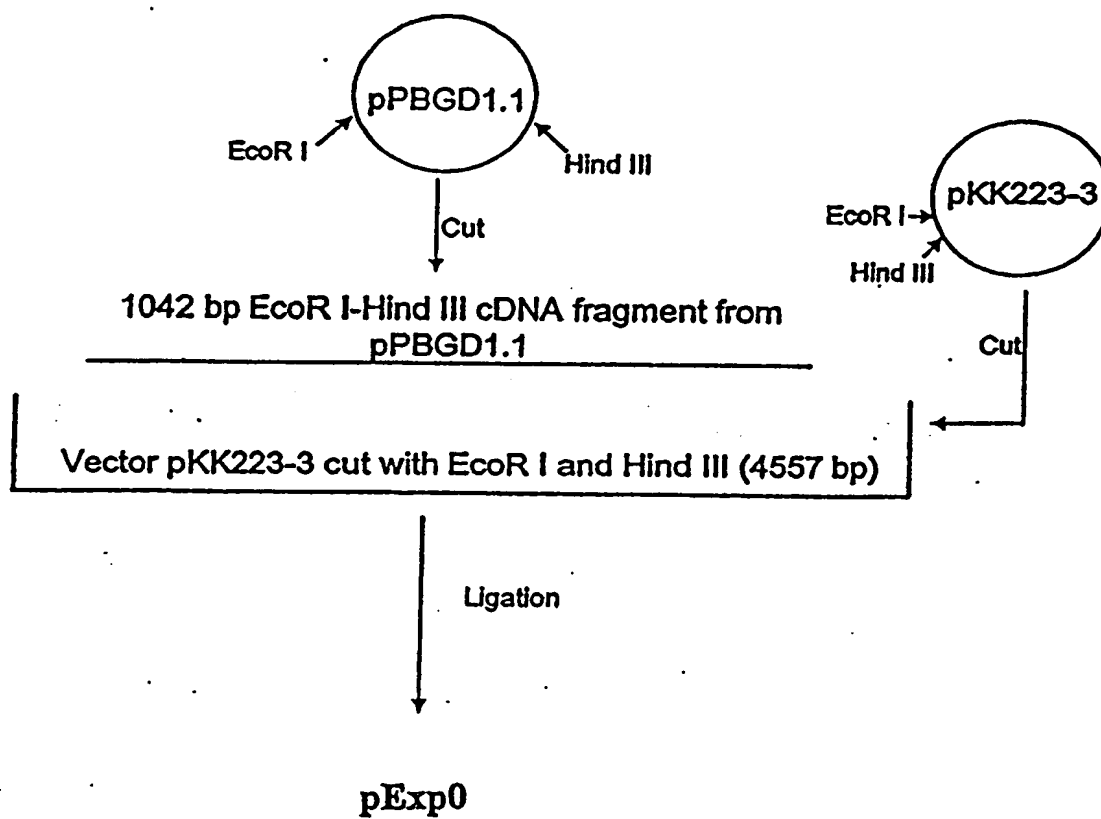


Fig. 1

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**Fig. 2**

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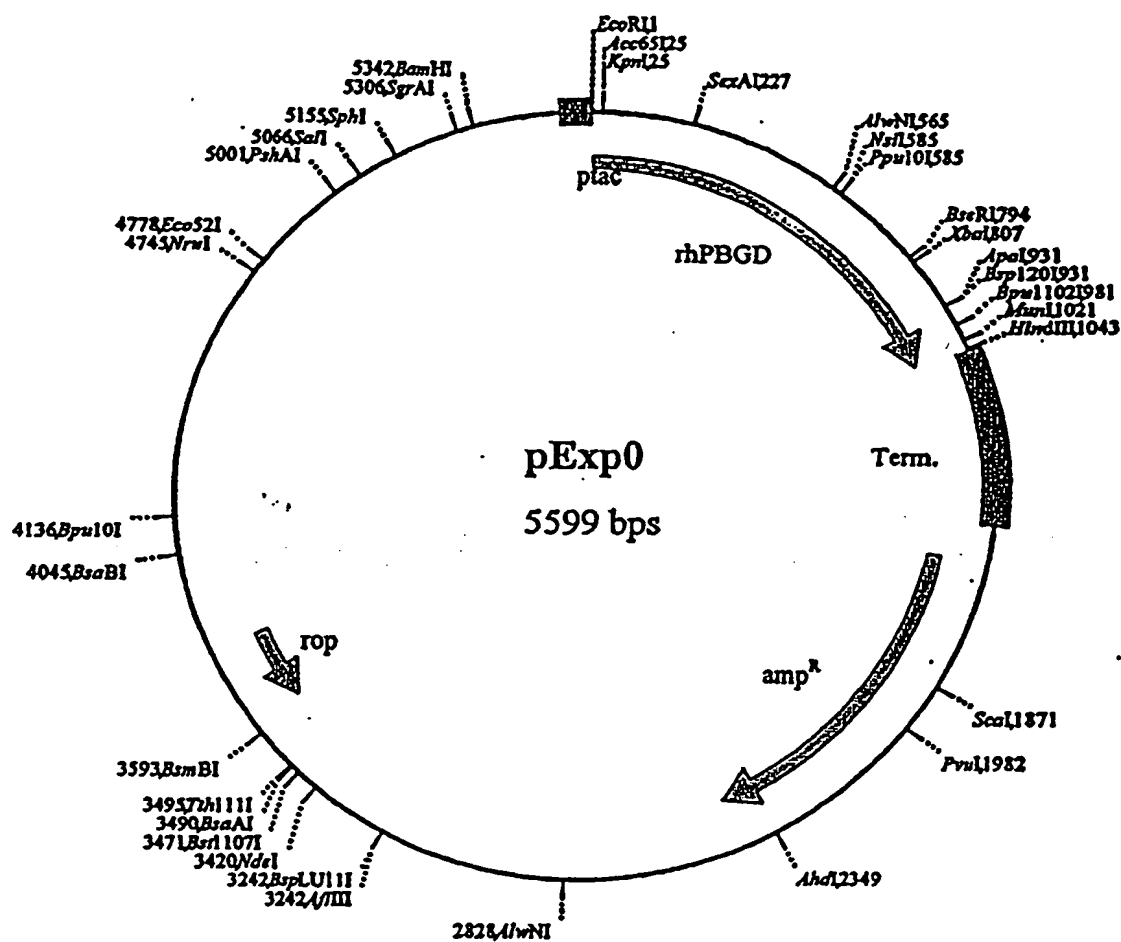


Fig. 3

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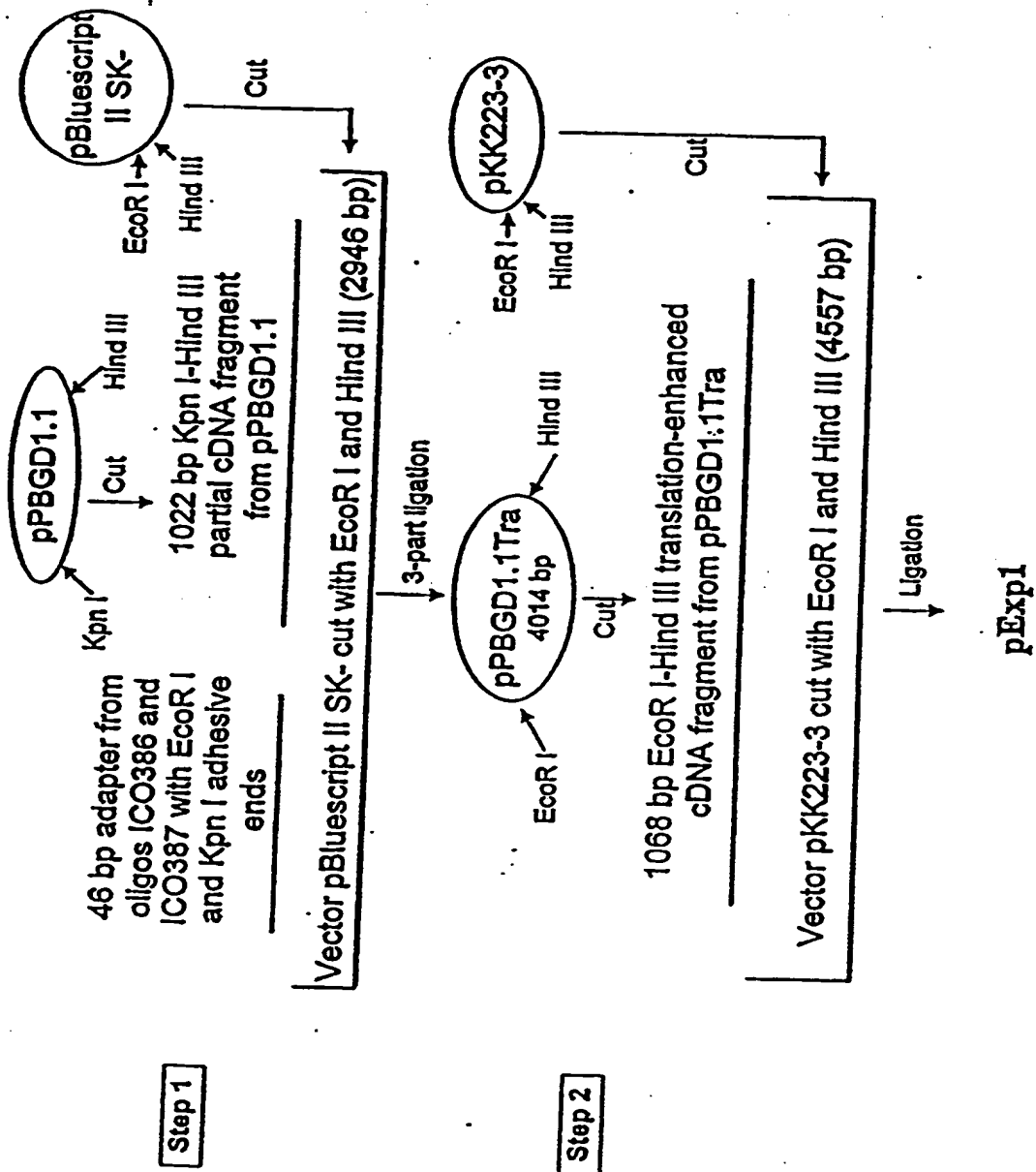


Fig. 4

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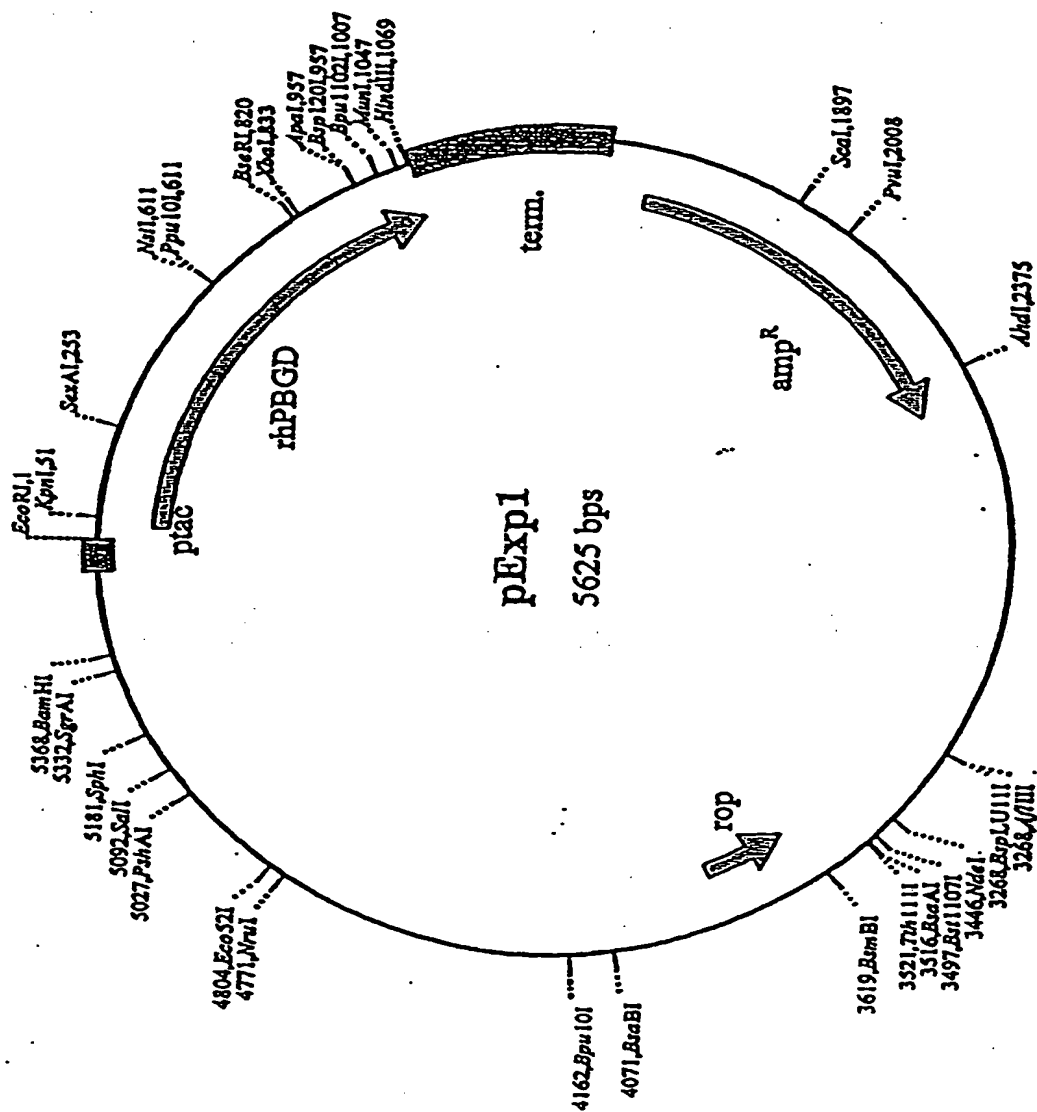
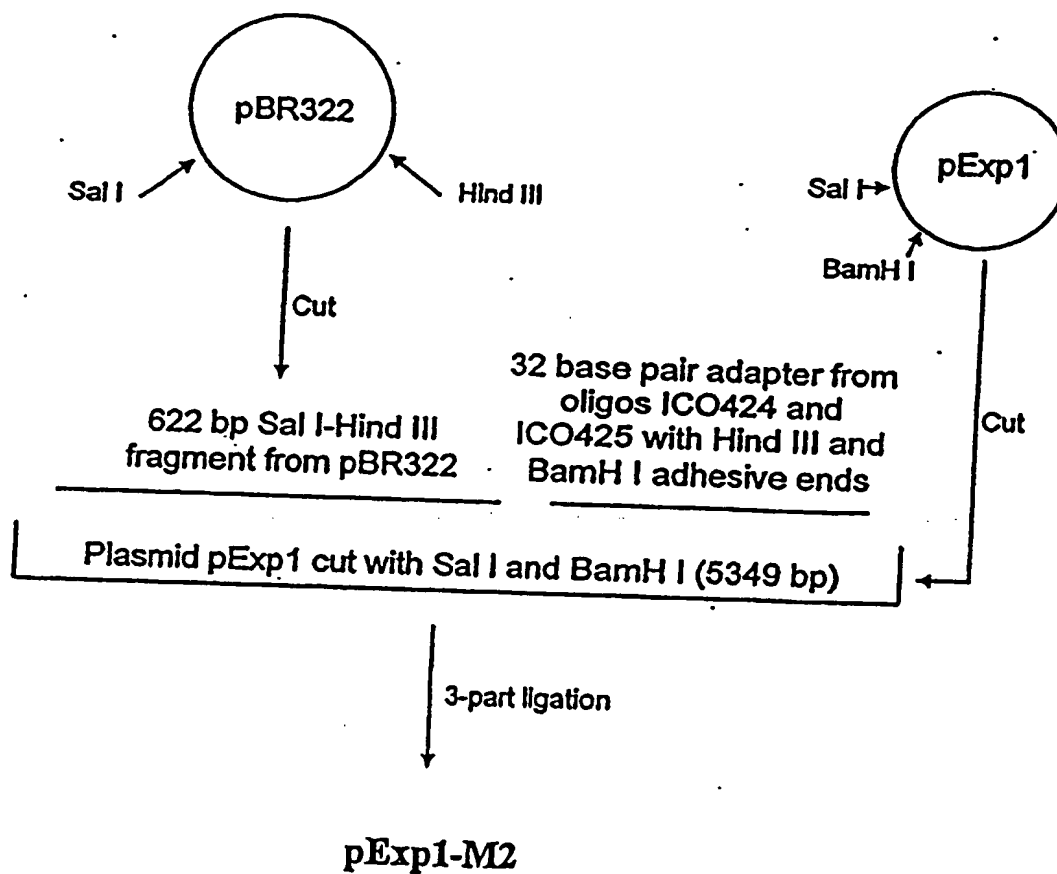
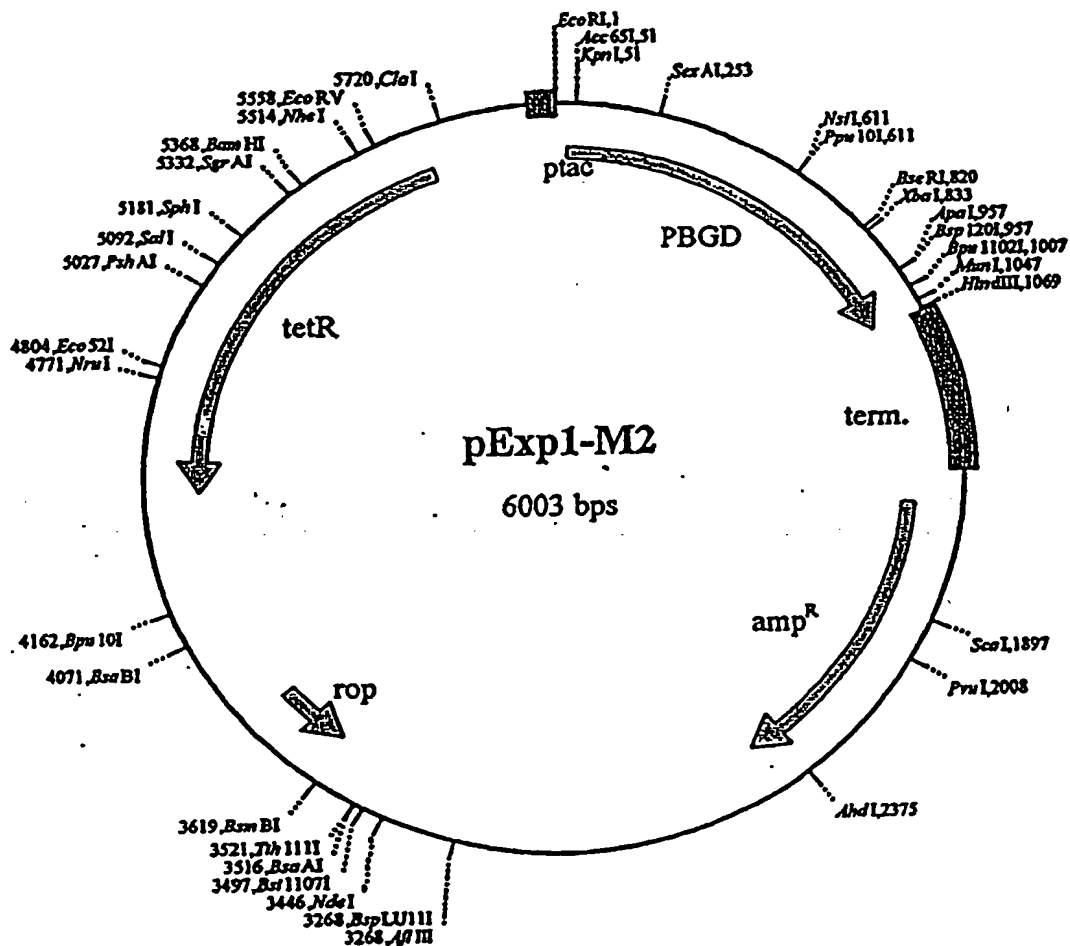


Fig. 5

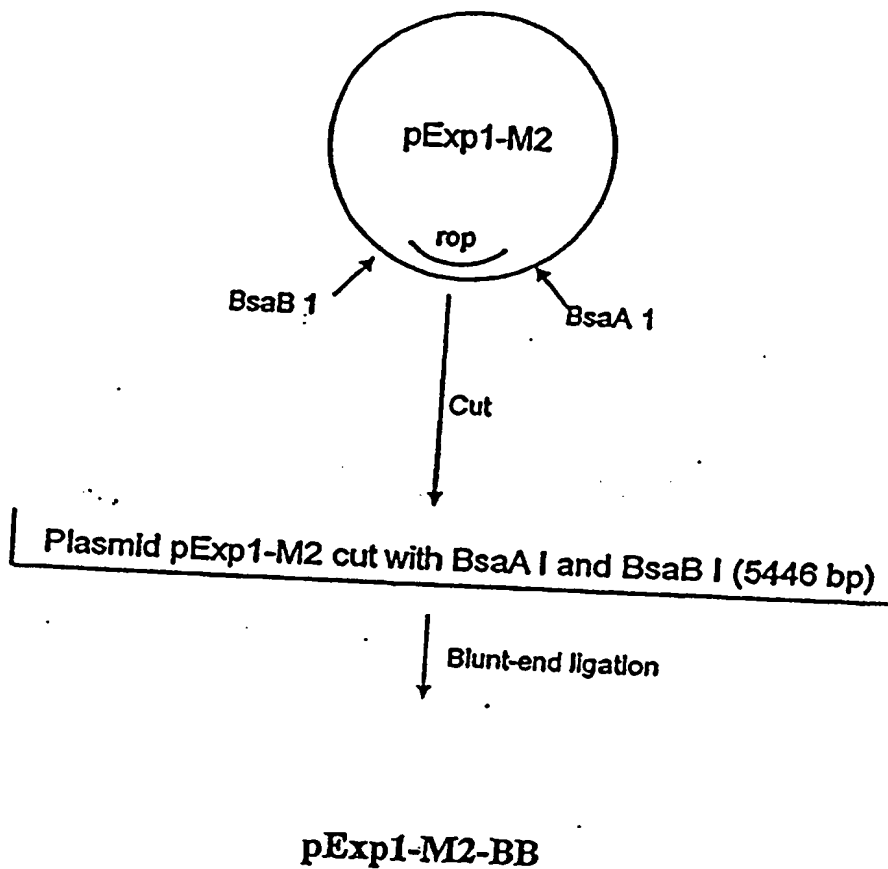
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**Fig. 6**

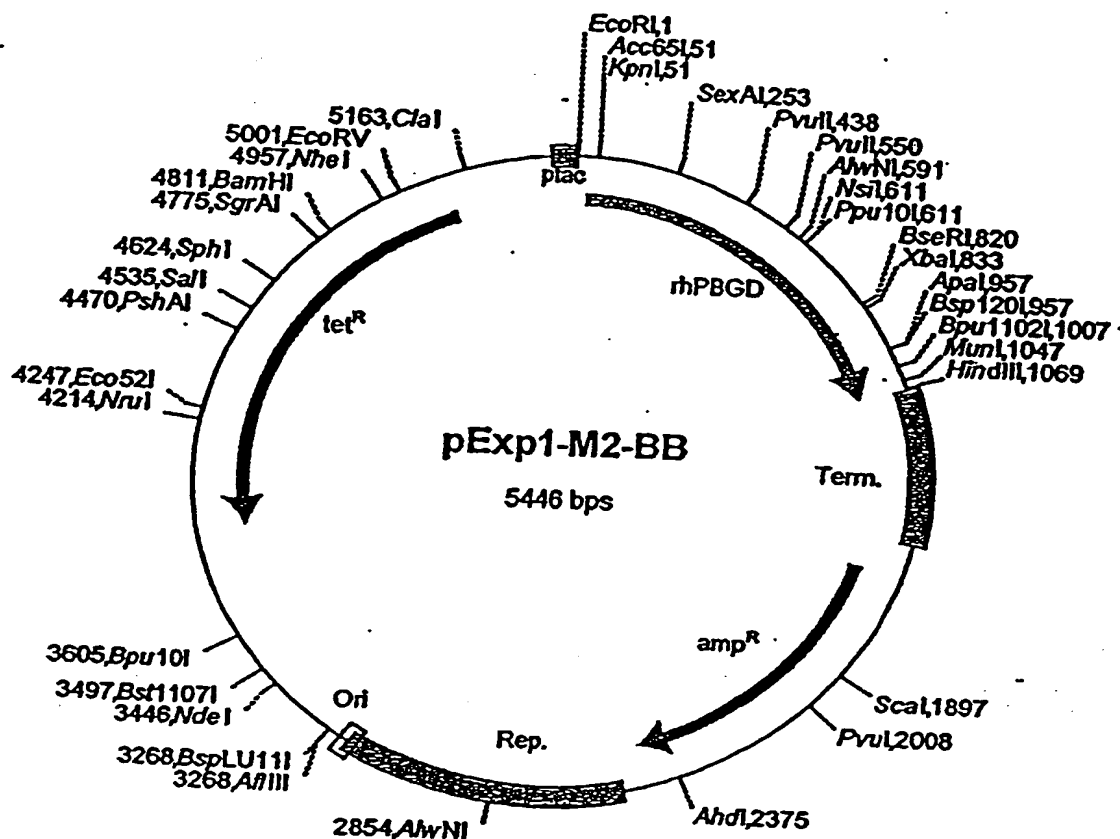




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**Fig. 8**

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## PLASMID FEATURES:

Start	End	Name
33	1067	rhPBGD (open reading frame)
1593	2453	amp <sup>R</sup> (open reading frame)
5105	3915	tet <sup>R</sup> (open reading frame)
5376	1	ptac (promoter)
1075	1501	Term. (terminator region)
3214	2519	Rep. (replication region)
3214		Ori (origin of replication)

Fig. 9

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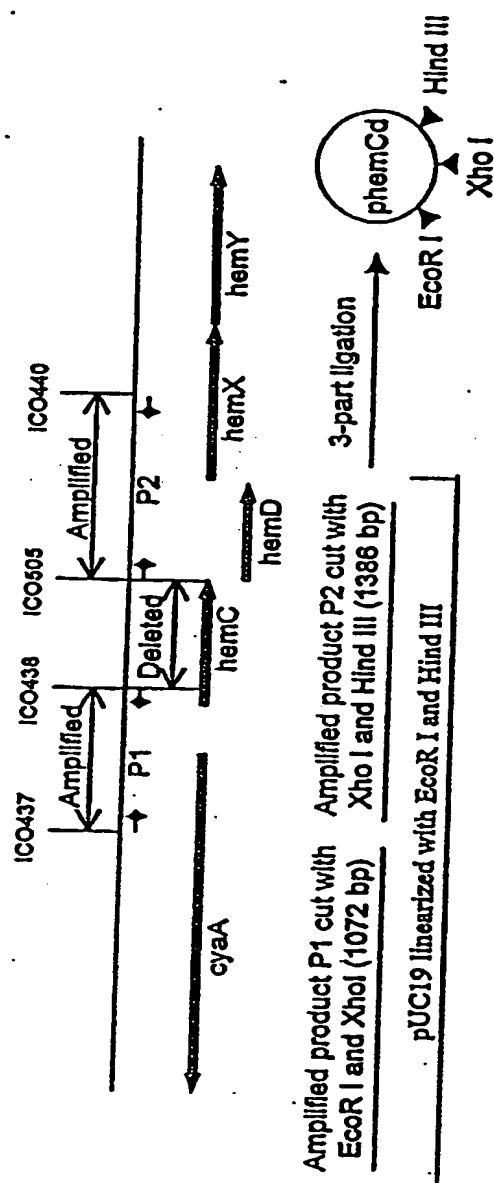


Fig. 10

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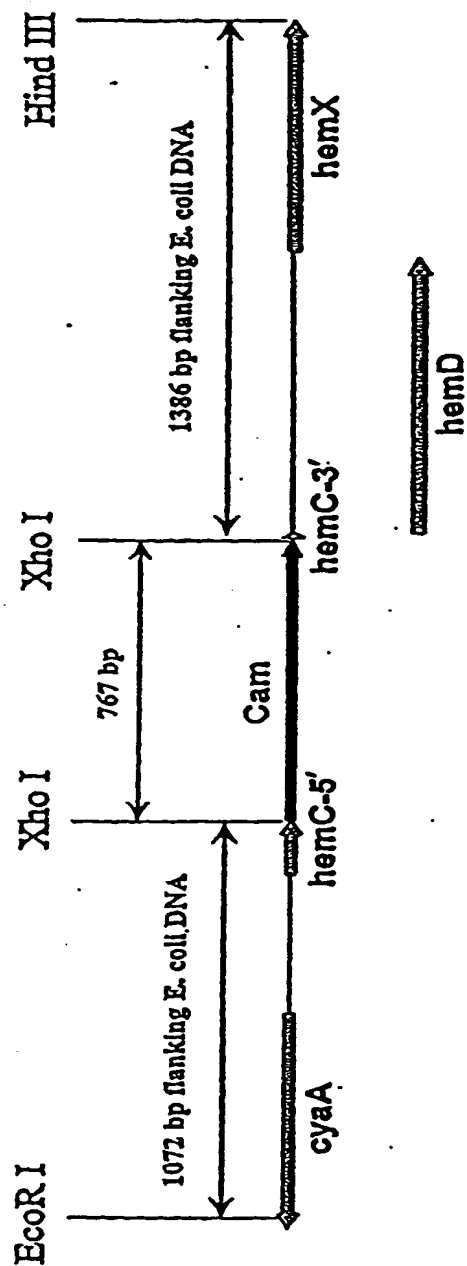


Fig. 11

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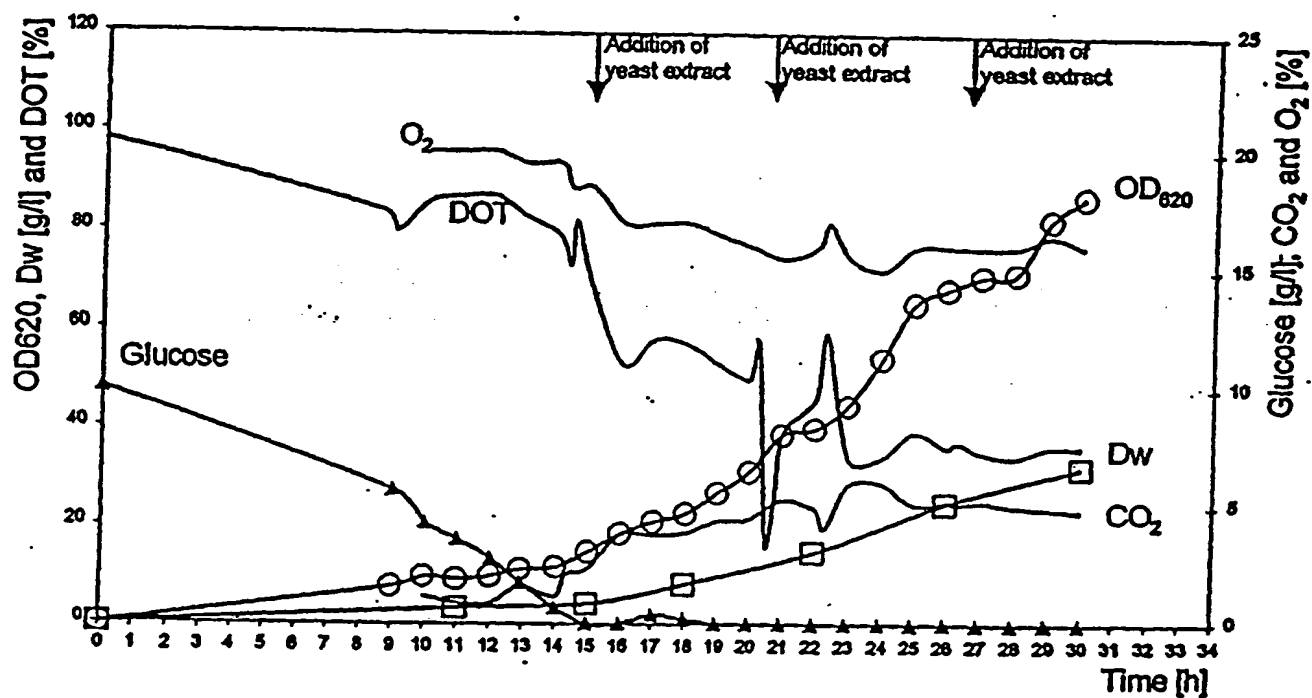


Fig. 12

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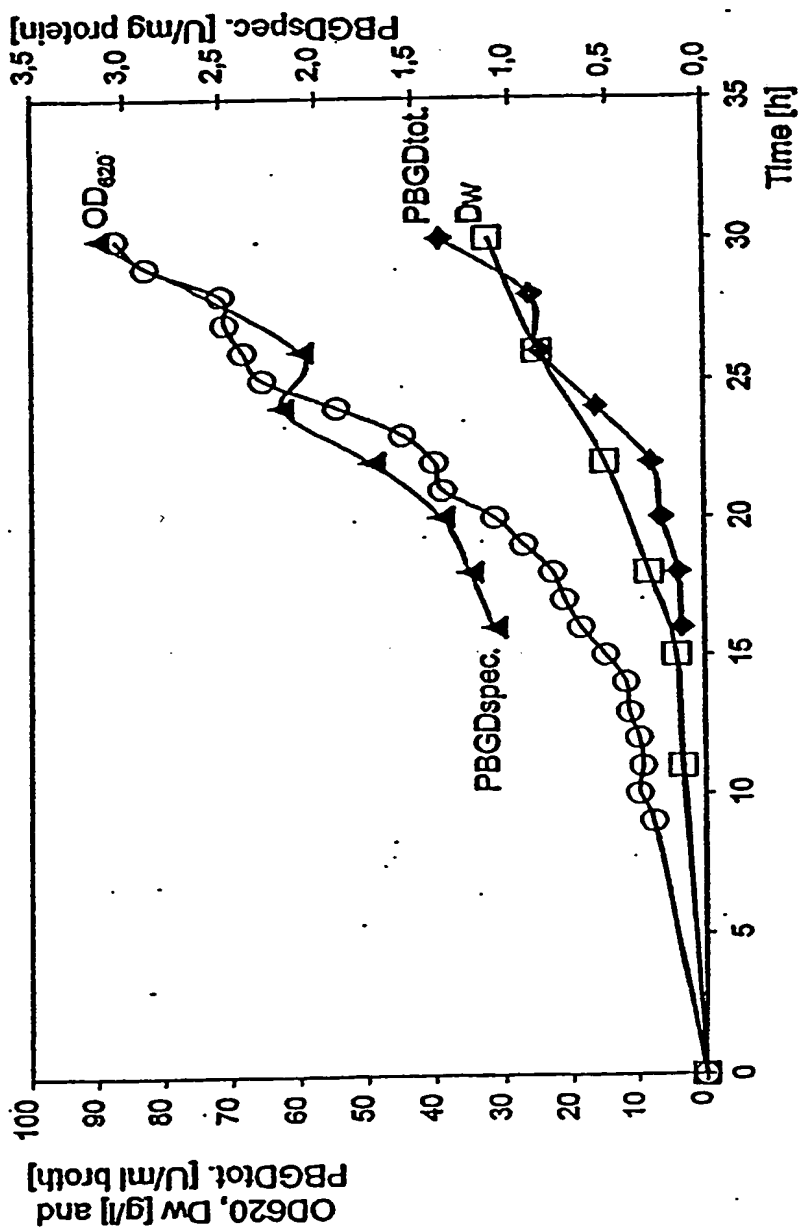
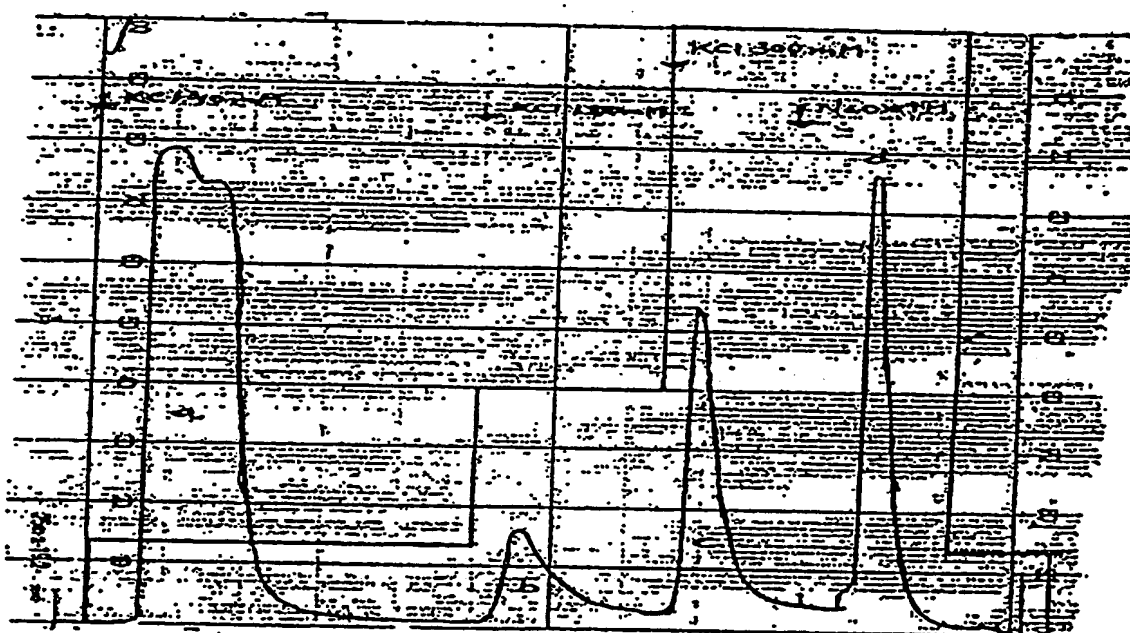


Fig. 13

Fig. 13

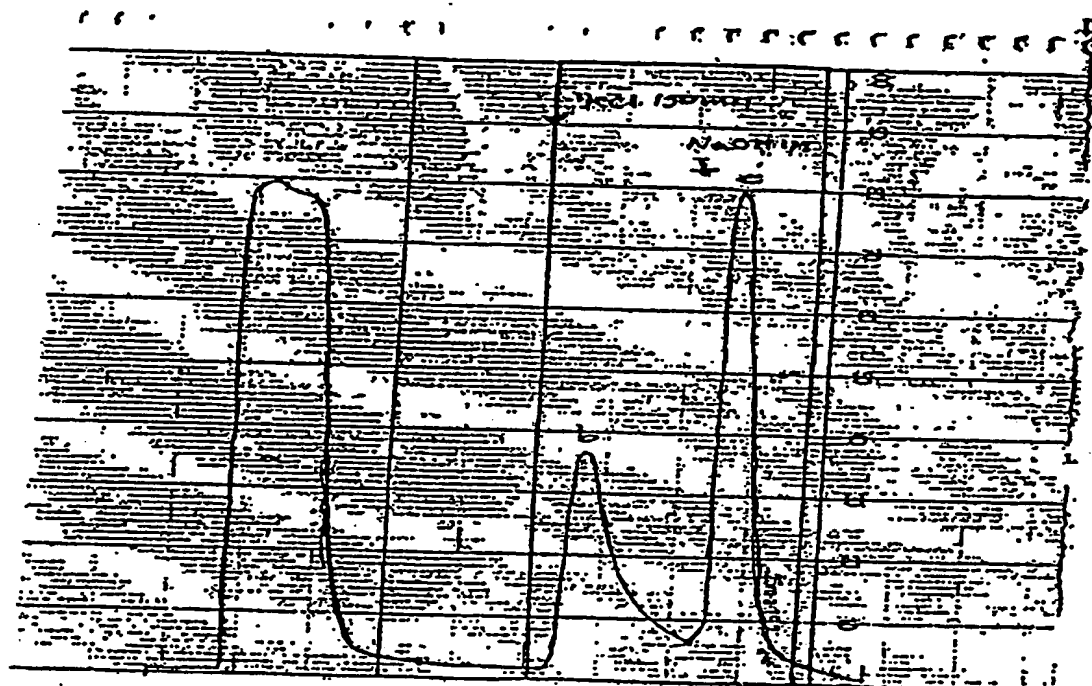
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Chromatography on DEAE-Sepharose FF (DEAE1). Peak a flow through the gel 40 mM KCl, Peak b 120 mM KCl Peak c 300 mM KCl Peak d NaOH 1 M.

**Fig. 14**

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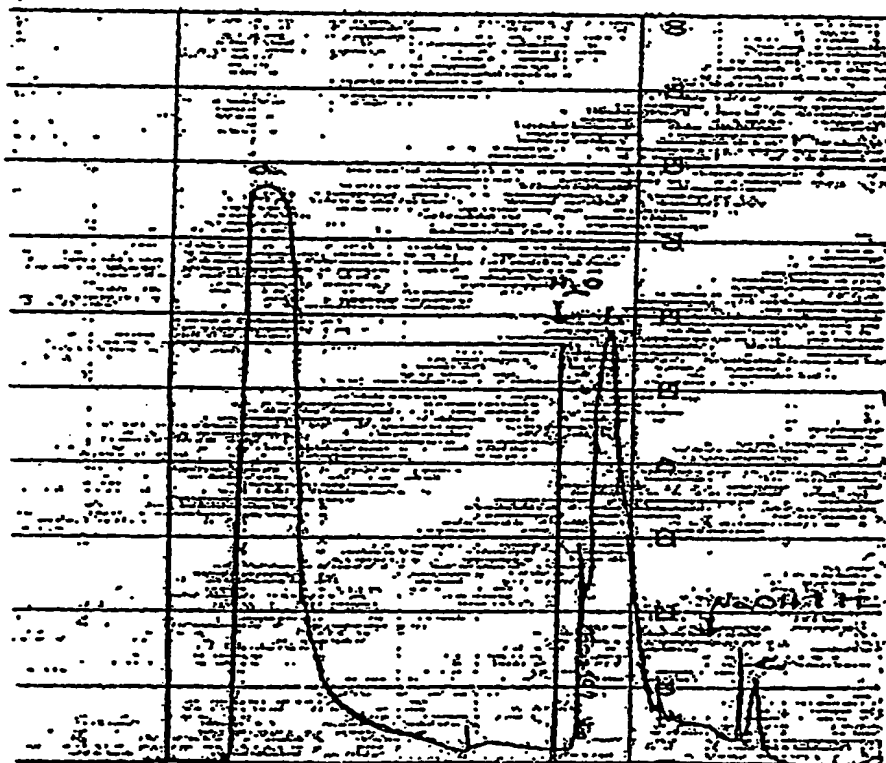


Chromatography on DEAE-Sepharose FF (DEAE2). Peak a flow through the gel 40 mM KCl, Peak b 150 mM KCl Peak c NaOH 1 M.

**Fig. 15**



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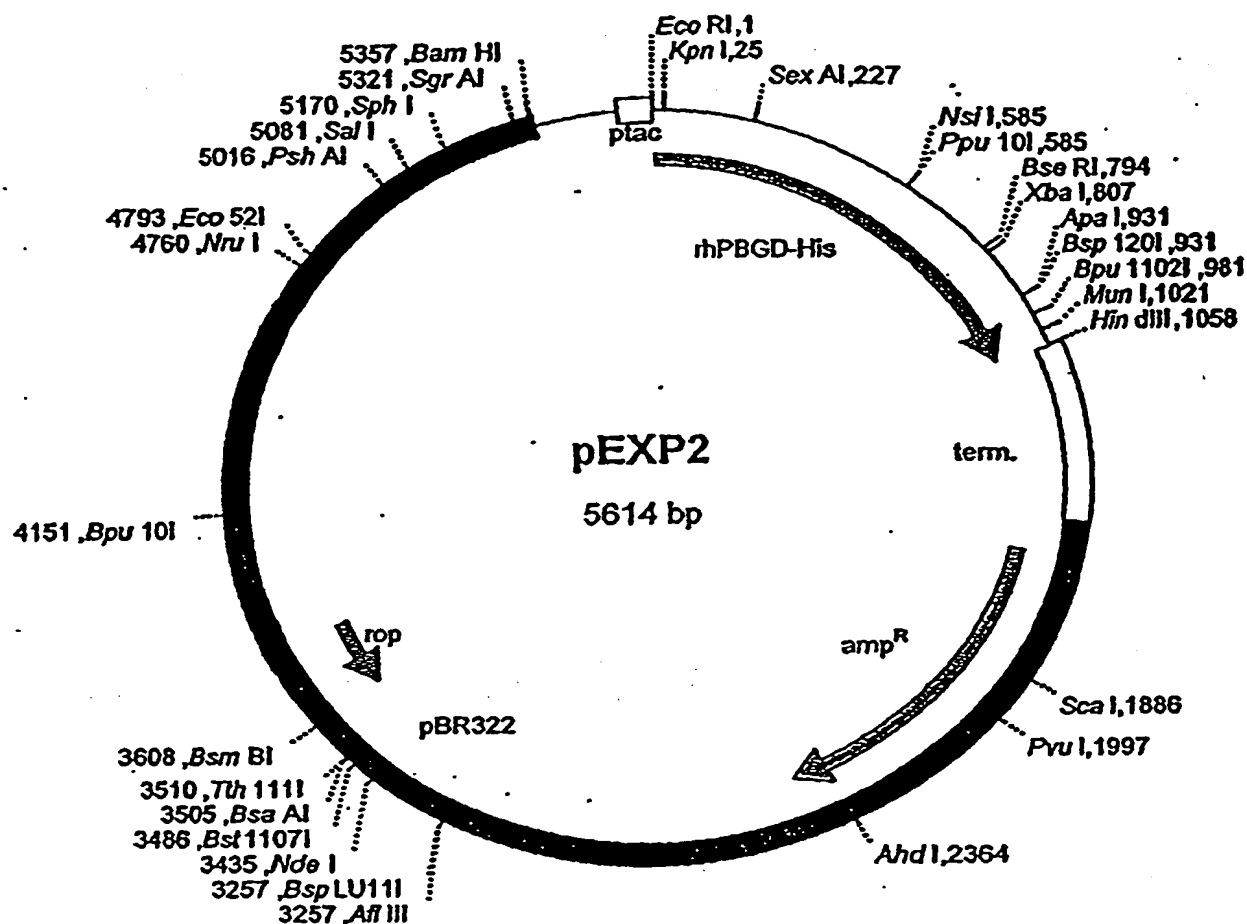


Chromatography on Butyl-Sepharose 4 FF. Peak a flow through the gel, Peak b water Peak c NaOH 1 M.

**Fig. 16**

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## PLASMID FEATURES:

Start	End	Name
7	1056	rhPBGD-His
1064	1490	terminator region
1582	2442	amp <sup>R</sup>
3820	3629	rop
5362	1490	pBR322
5544	1	ptac (promoter)

Fig. 17

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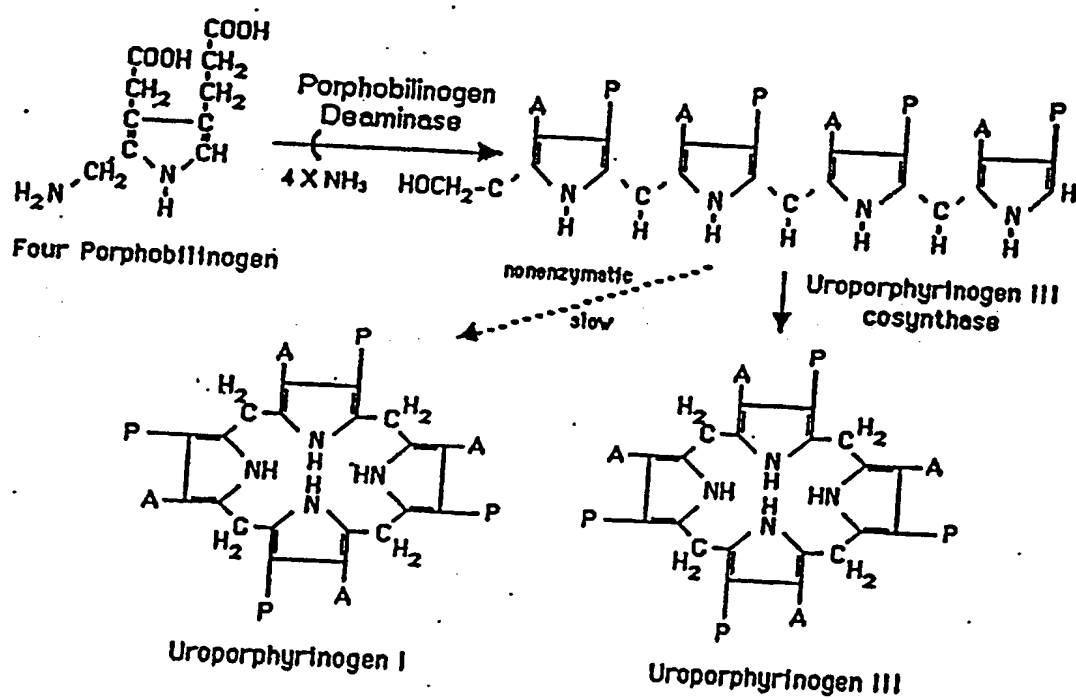


Fig. 18

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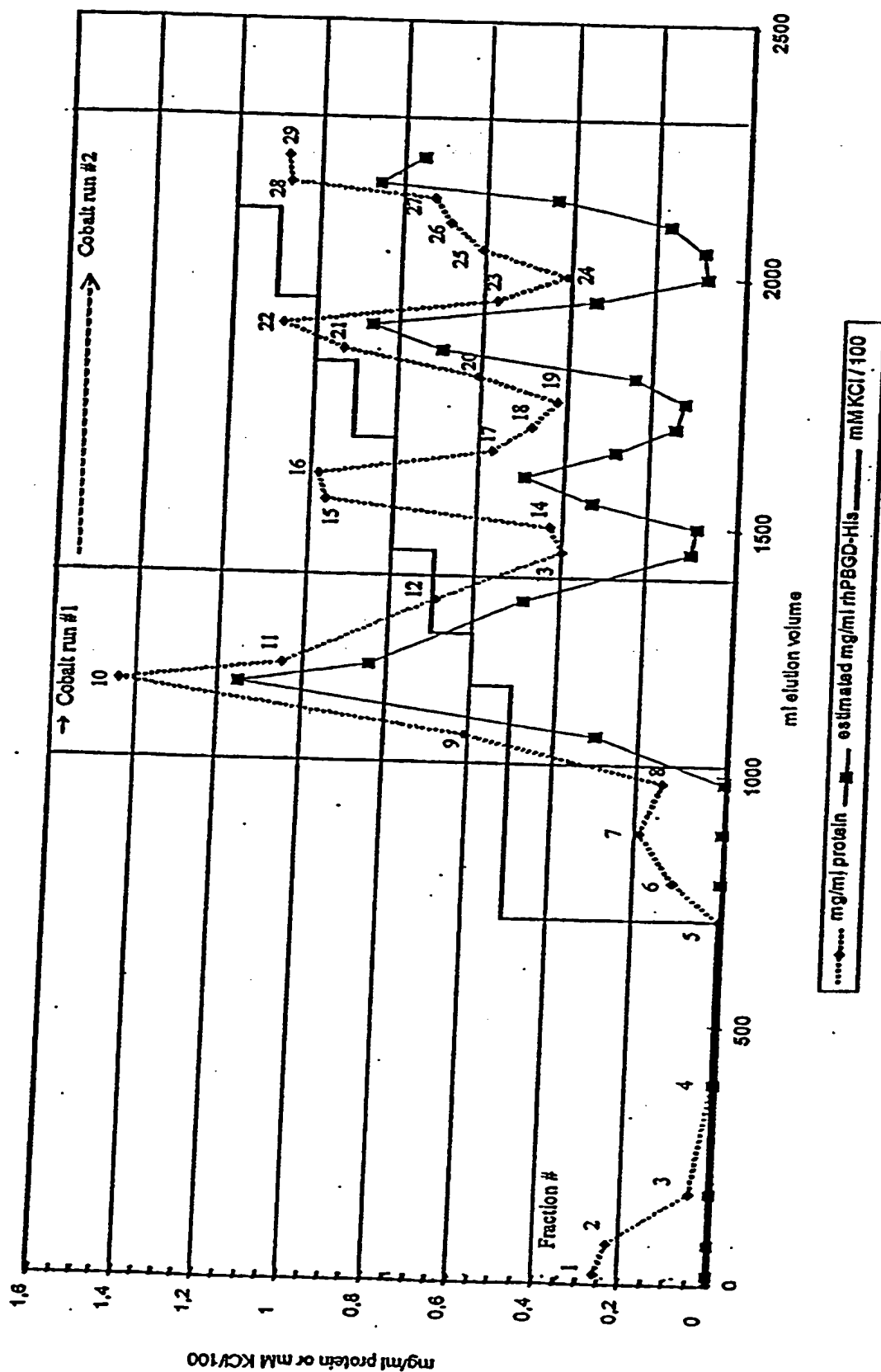
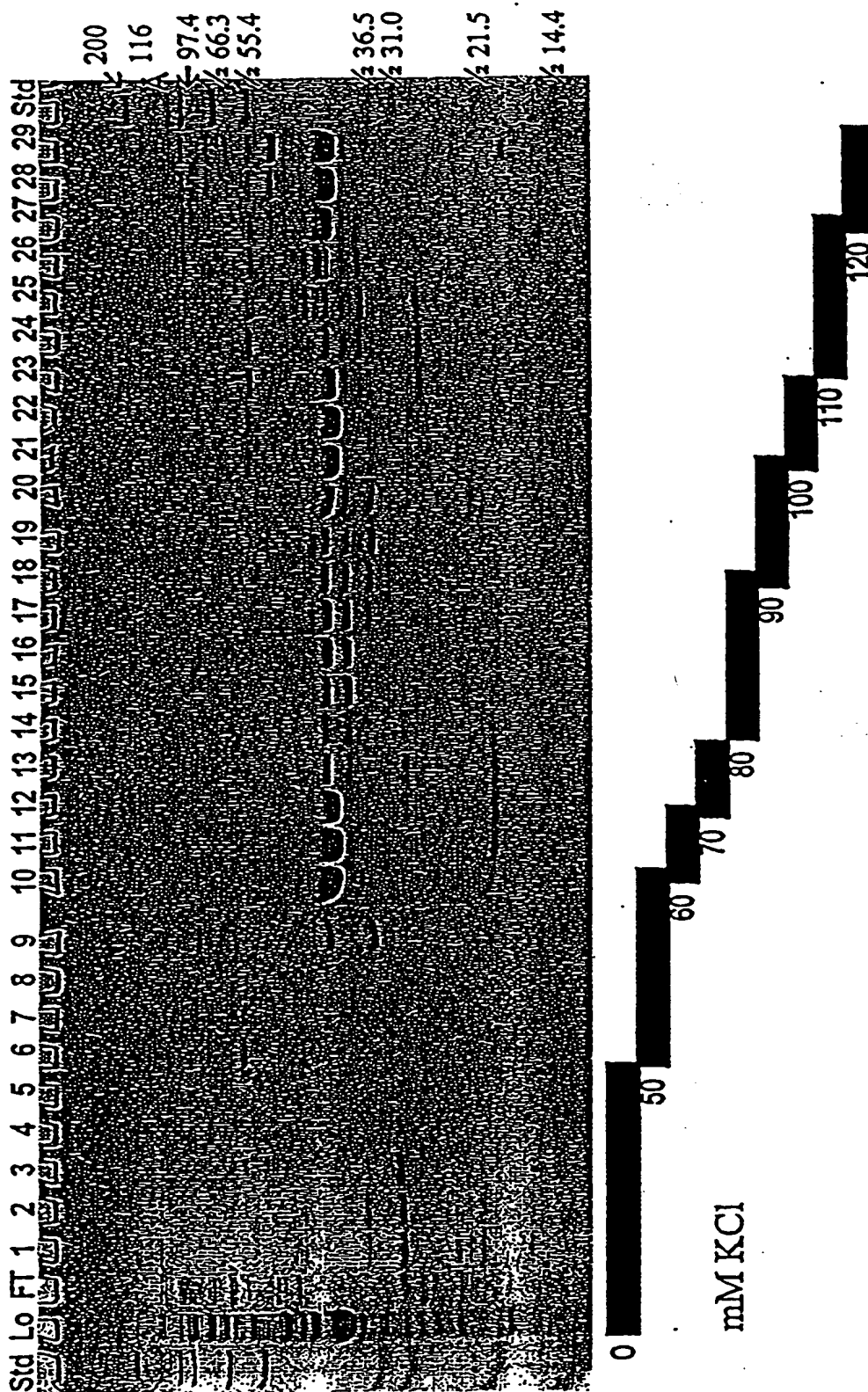


Fig. 19

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# DEAE Sepharose Column Fractions:



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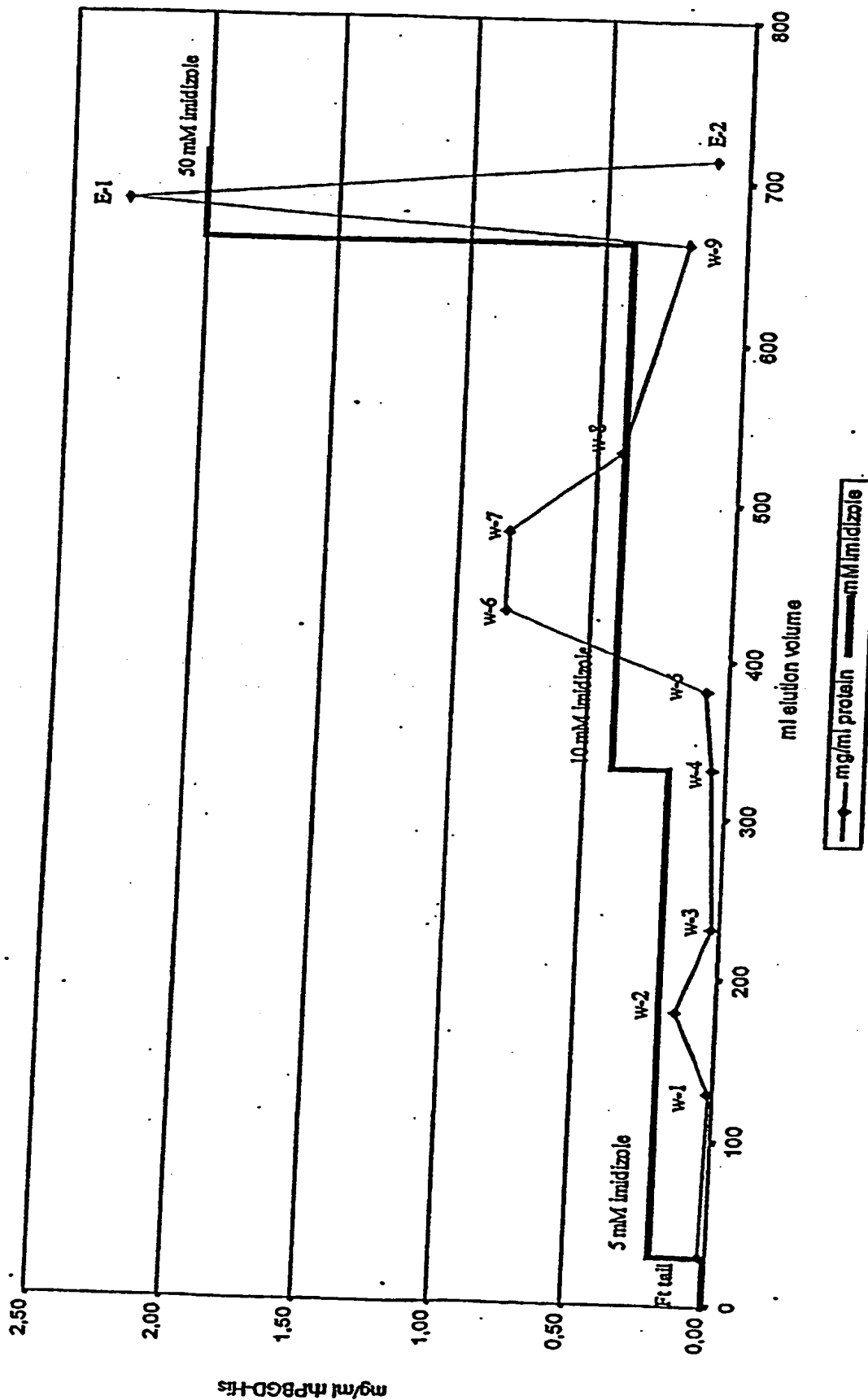
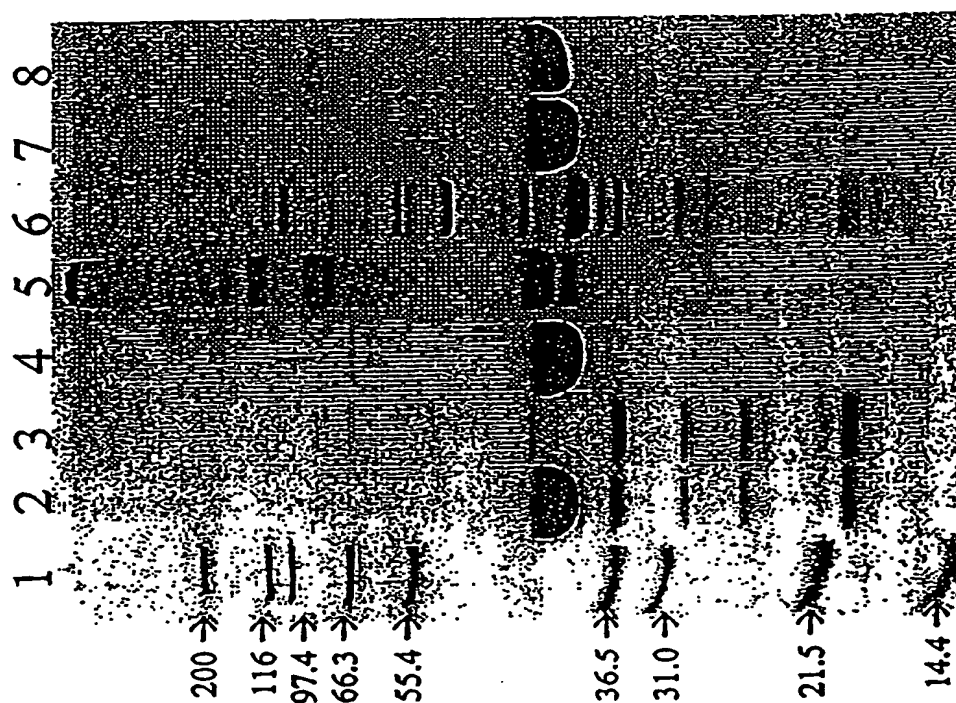


Fig. 21

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1	Mark 12 molecular weight standards (Novex)
2	Cobalt load #1 (DEAE fractions 9 → 12)
3	Flowthrough from cobalt load #1
4	50 mM imidazole eluate from cobalt run #1
5	Concatamerized nickel purified rhPBGD-His
6	Flowthrough from cobalt load #2
7	10 mM imidazole eluate from cobalt run #2
8	50 mM imidazole eluate from cobalt run #2

Fig. 22

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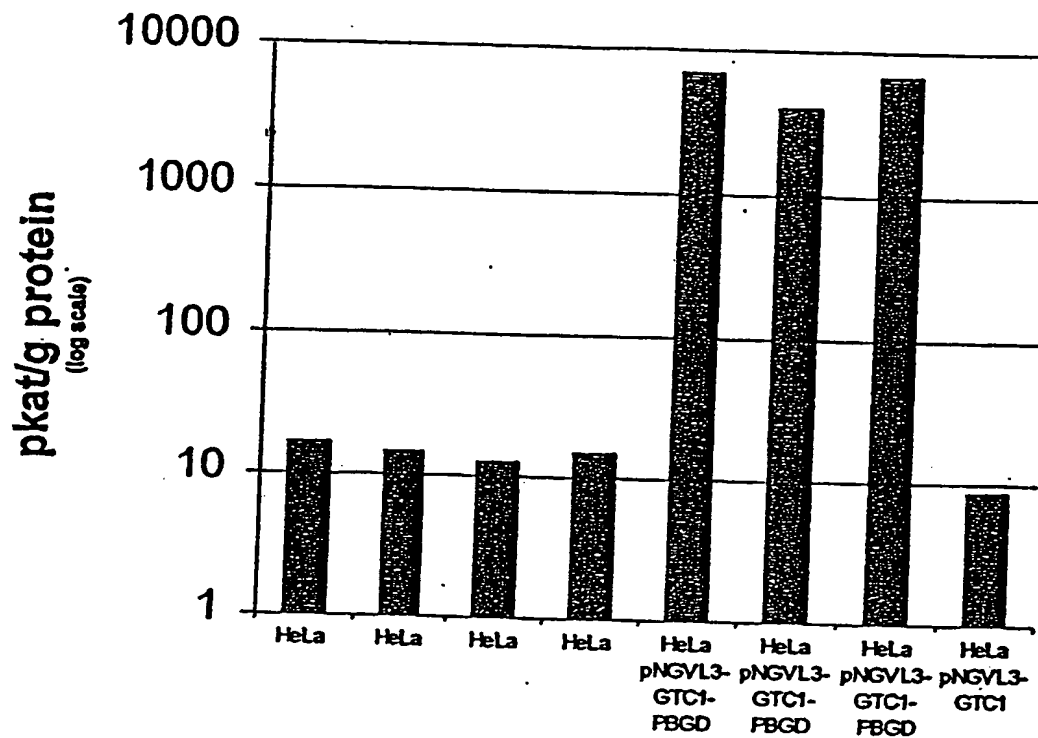


Fig 23: PBGD activity related to protein concentration in HeLa cells.

**Fig. 23**

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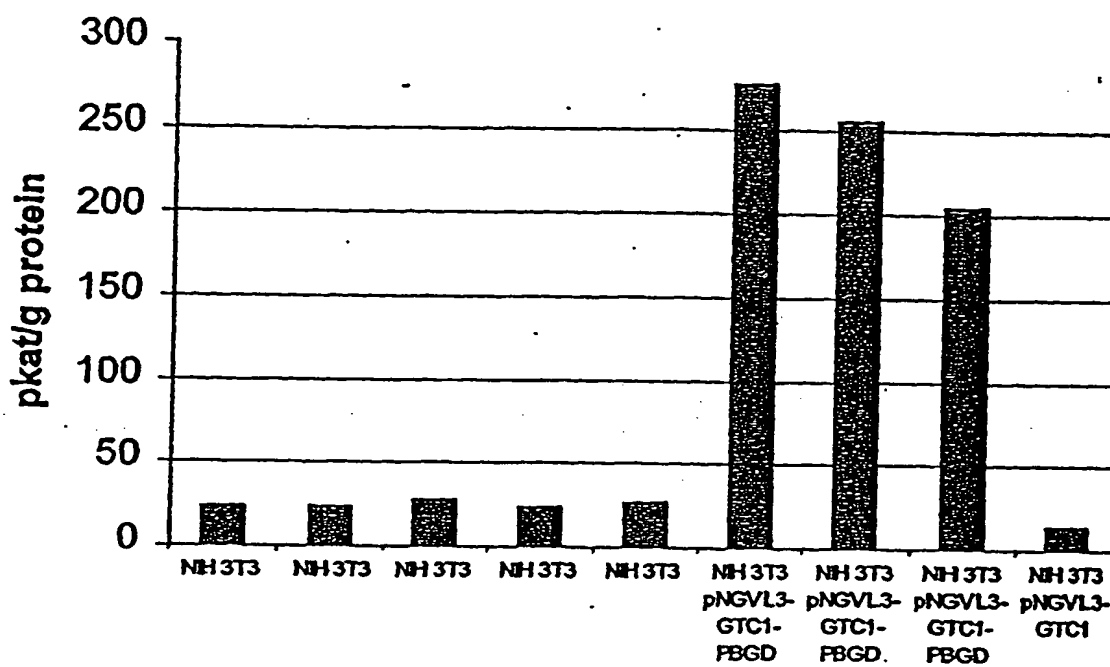


Figure 24: PBGD activity related to protein concentration in NIH 3T3 cells.

**Fig. 24**

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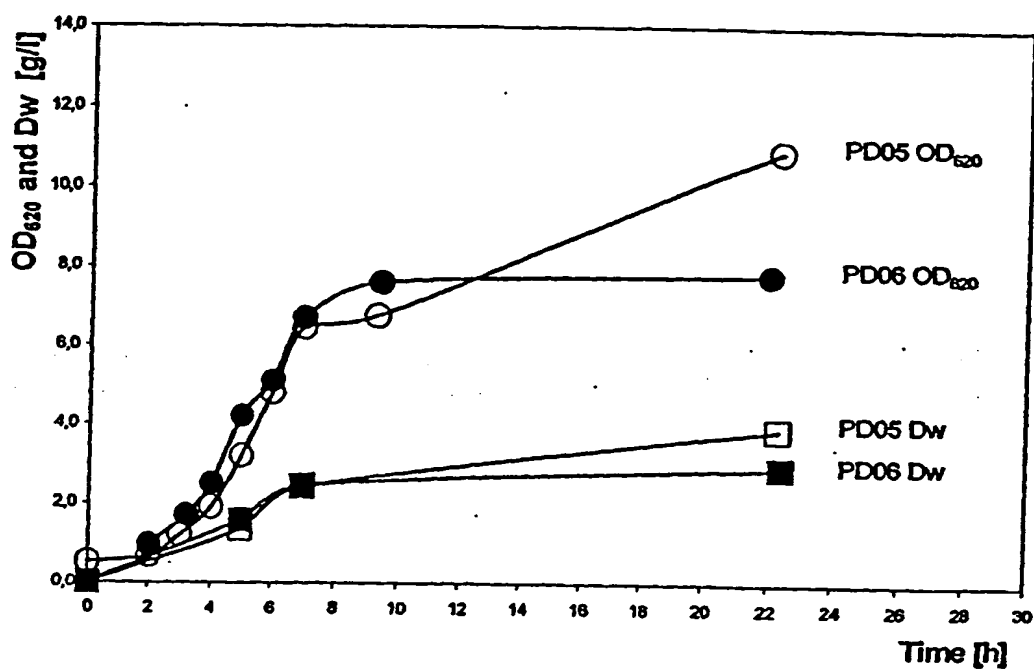


Fig 25. Comparison of fermentations PD05 and PD06 with strain PBGD-2.

**Fig. 25**

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Fig 26. Comparison of fermentations PD09, PD11 and PD12

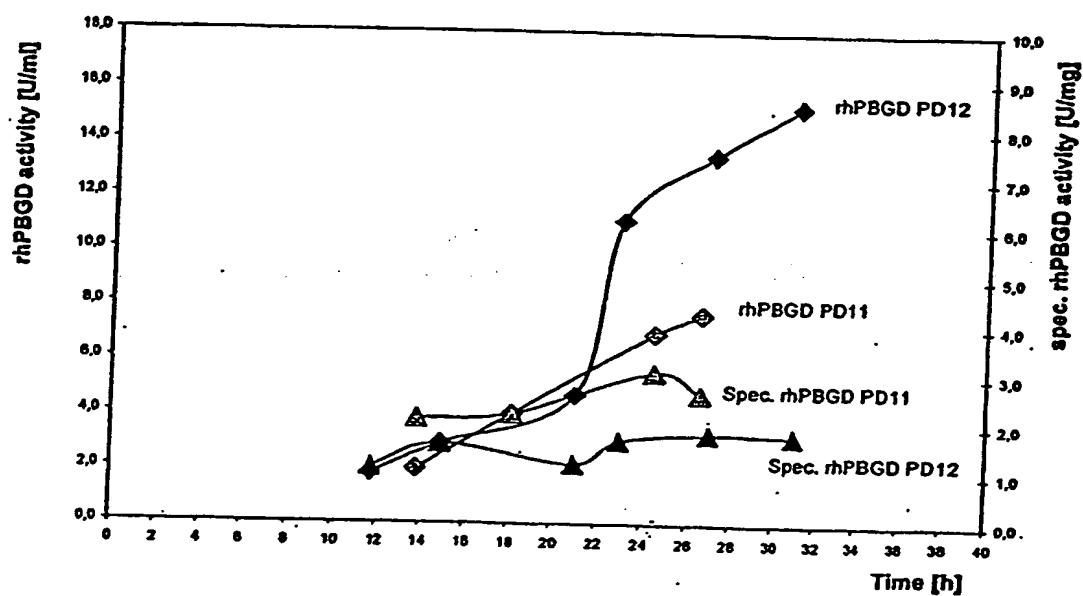


Fig. 26

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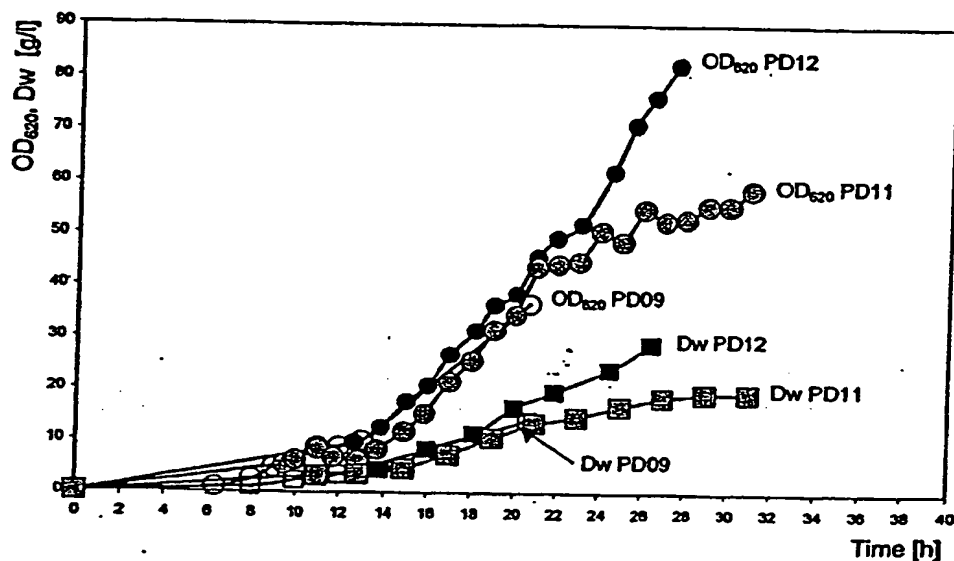


Fig 27: Comparison of fermentations PD09, PD11 and PD12 with strain PBGD-1.

**Fig. 27**

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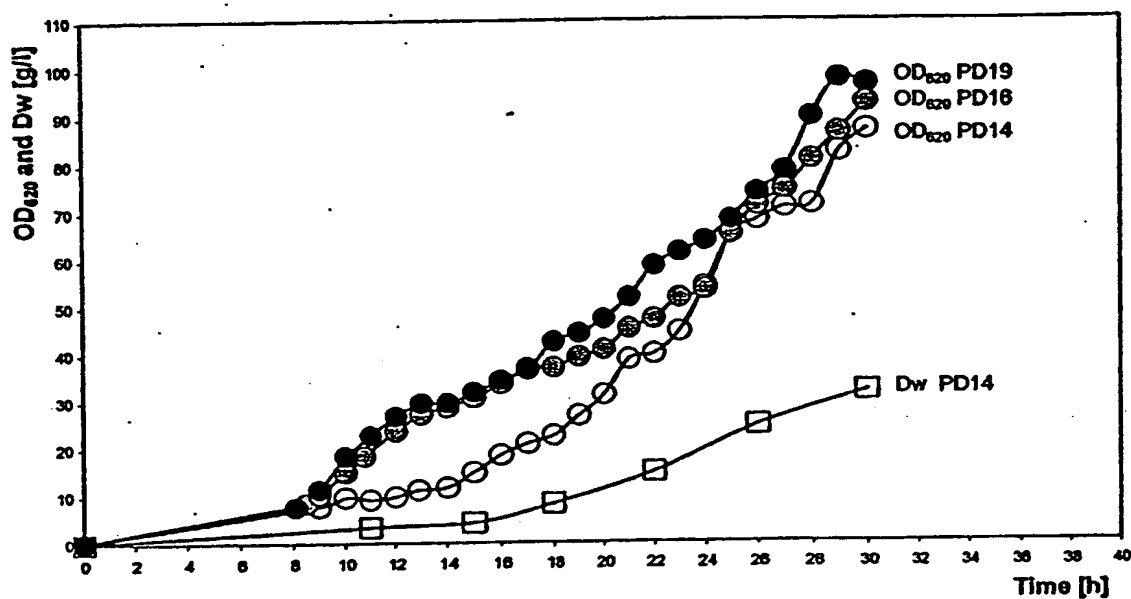


Fig 28. Comparison of fermentations PD14, PD16 and PD19 with strain PBGD-2.

**Fig. 28**

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Fig 29. Comparison of fermentations PD14, PD16 and PD19 with strain PBGD-2.

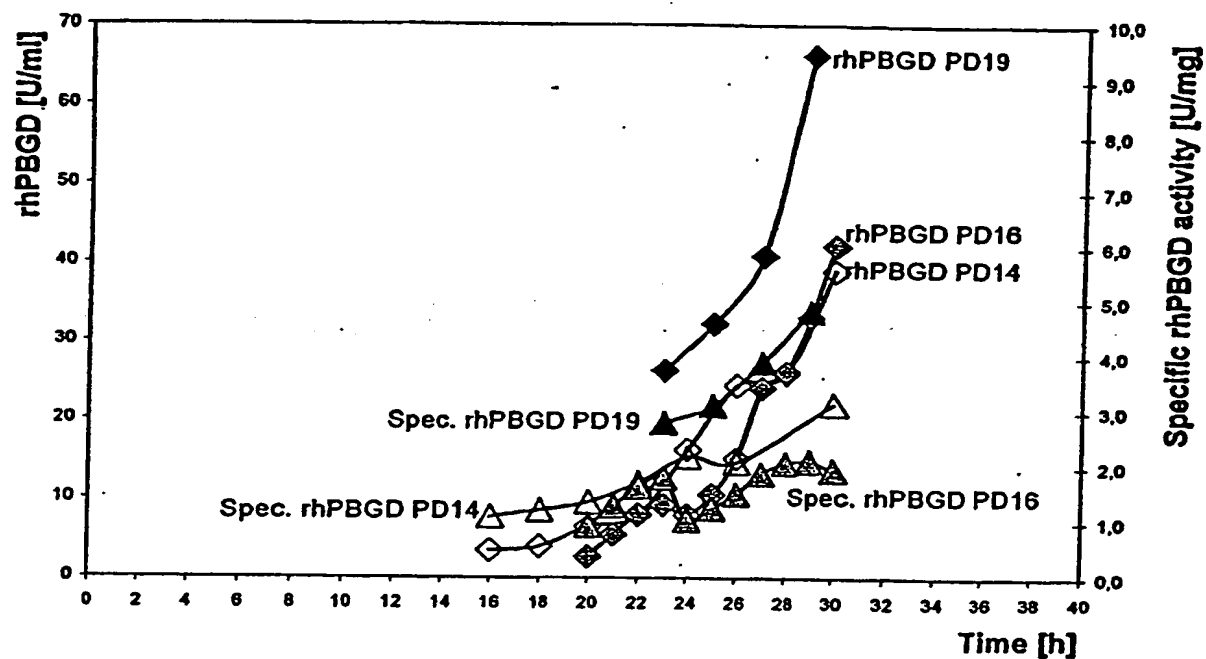


Fig. 29

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Fig 30. Comparison of fermentations PD19, PD21 and PD22 with strain PBGD-2.

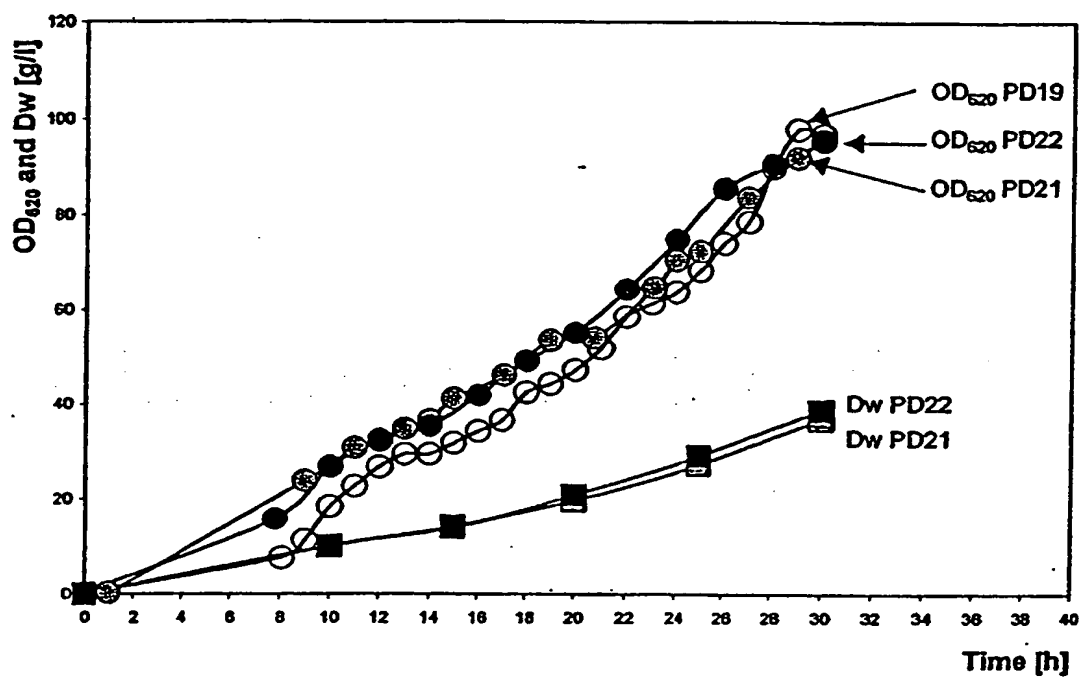


Fig. 30

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Fig 31. Comparison of fermentations PD19, PD21 and PD22 with strain PBGD-2.

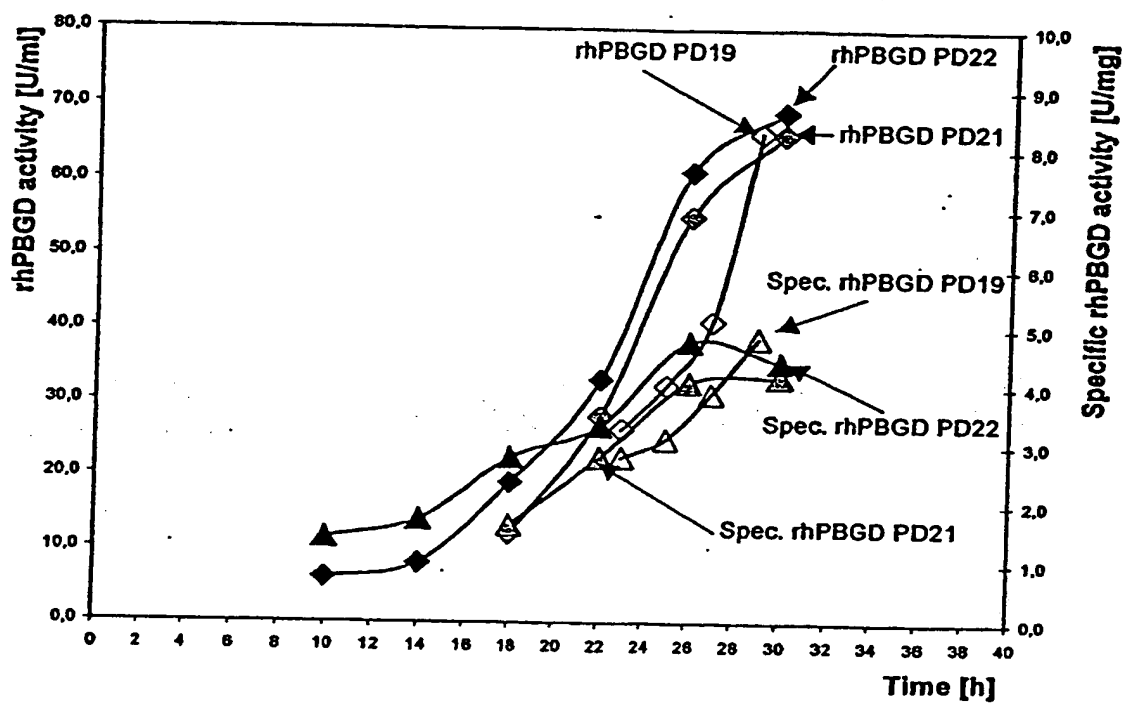


Fig. 31



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Fig 32. Comparison of fermentations PD19, PD1501 and PD1502.

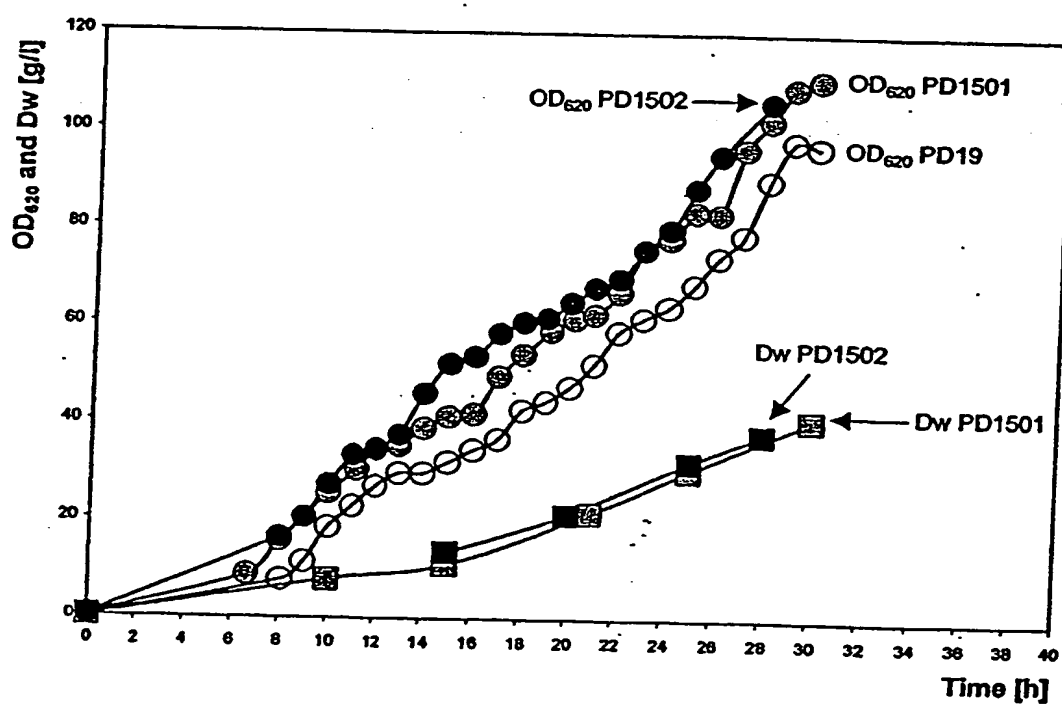


Fig. 32

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Fig 33. Comparison of fermentations PD19, PD1501 and PD1502 with strain PBGD-2.

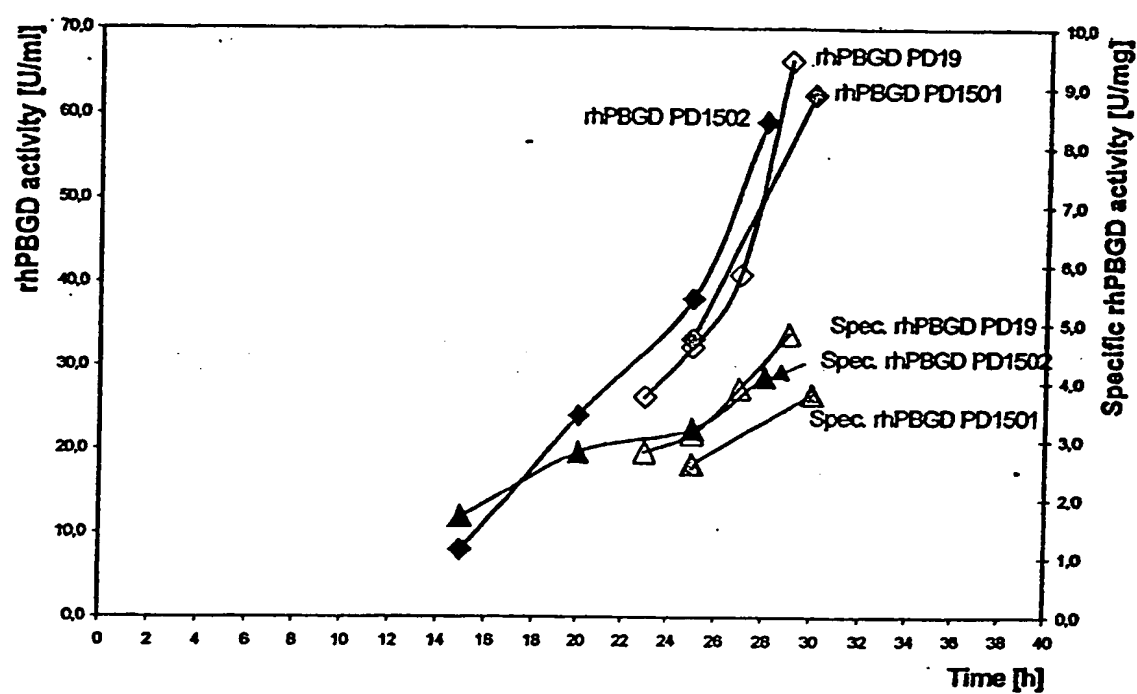
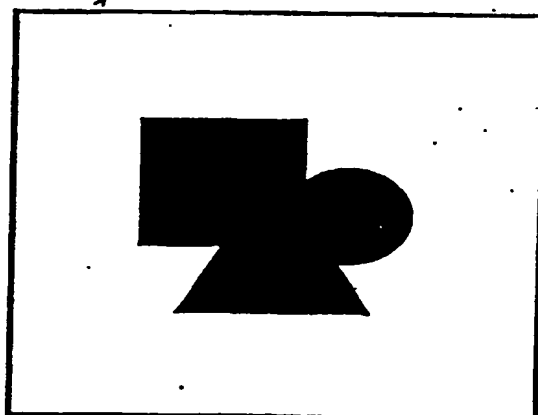


Fig. 33

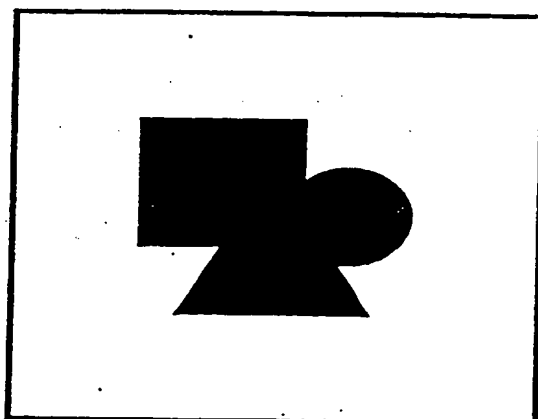
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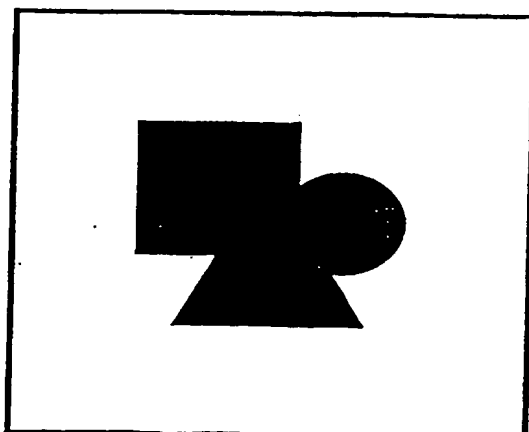
Summary of Fermentation and down stream process visualized by SDS-Page.  
Comparison between different samples.



Well	Samples from PD22
1	Mark 12 MW st
2	Extract
3	Homogenate
4	Broth 30 h
5	Broth 26 h
6	Broth 22 h
7	Broth 18 h
8	Broth 14 h
9	Broth 10 h
10	rhPBGD-His



	Samples from PD1501
1	Mark 12 MW st
2	Extract
3	Extract
4	Extract
5	Broth 30 h
6	Broth 30 h
7	Broth 25 h
8	Broth 20 h
9	Broth 15 h
10	rhPBGD-His



	Samples from PD1502
1	Mark 12 MW st
2	Broth 15 h
3	Broth 20
4	Broth 25 h
5	Broth 28 h
6	Homogenate
7	Extract
8	Extract
9	Extract
10	rhPBGD-His

Fig. 34

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Fig 35. Stability studies: Single use aliquots of extract were routinely taken out of the freezer (-20°C) and the rhPBGD-activity was measured and plotted over time.

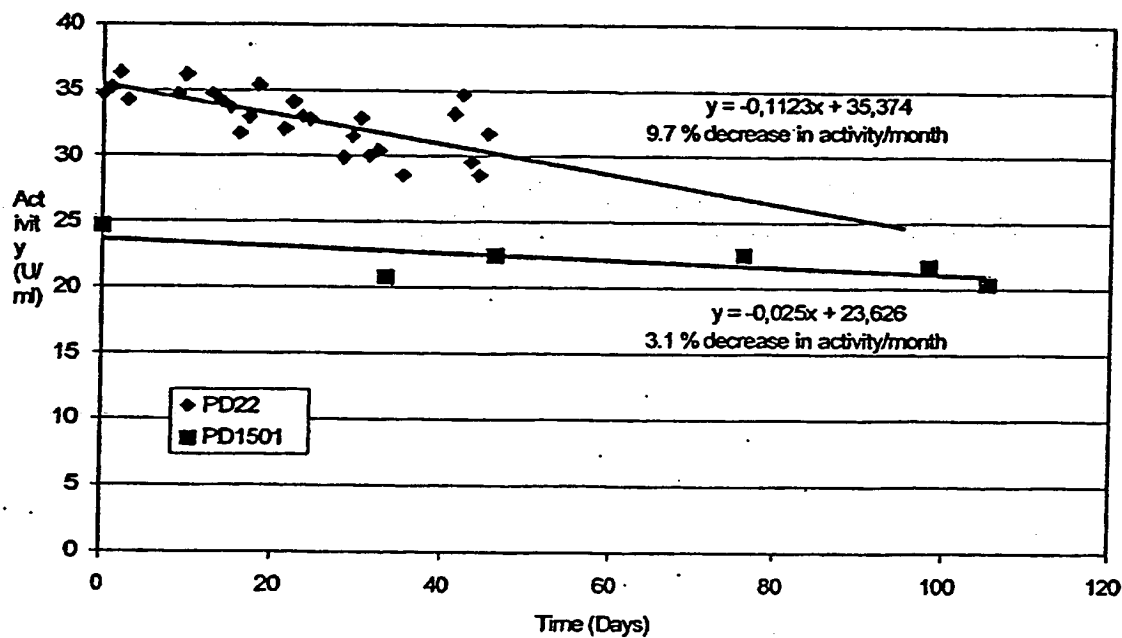
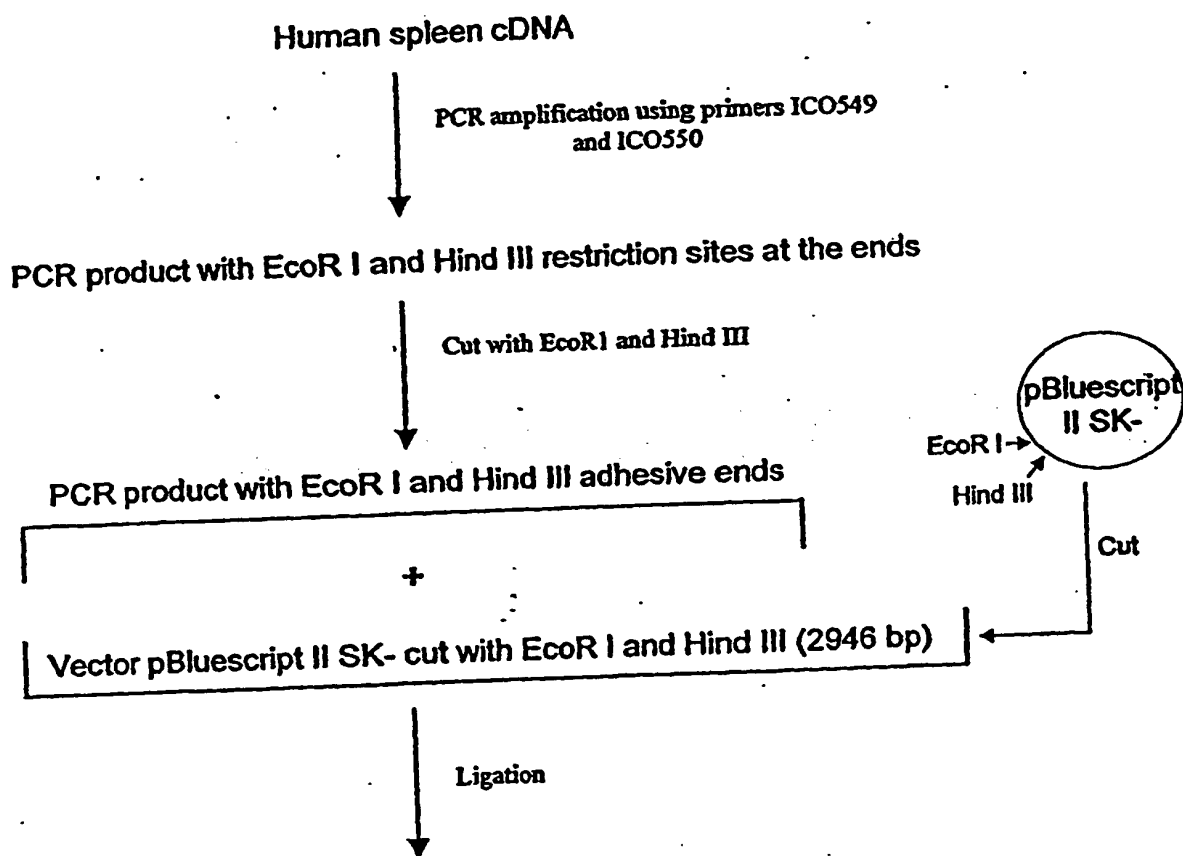


Fig. 35



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**Strategy for PCR cloning of ALAD****Fig. 37**

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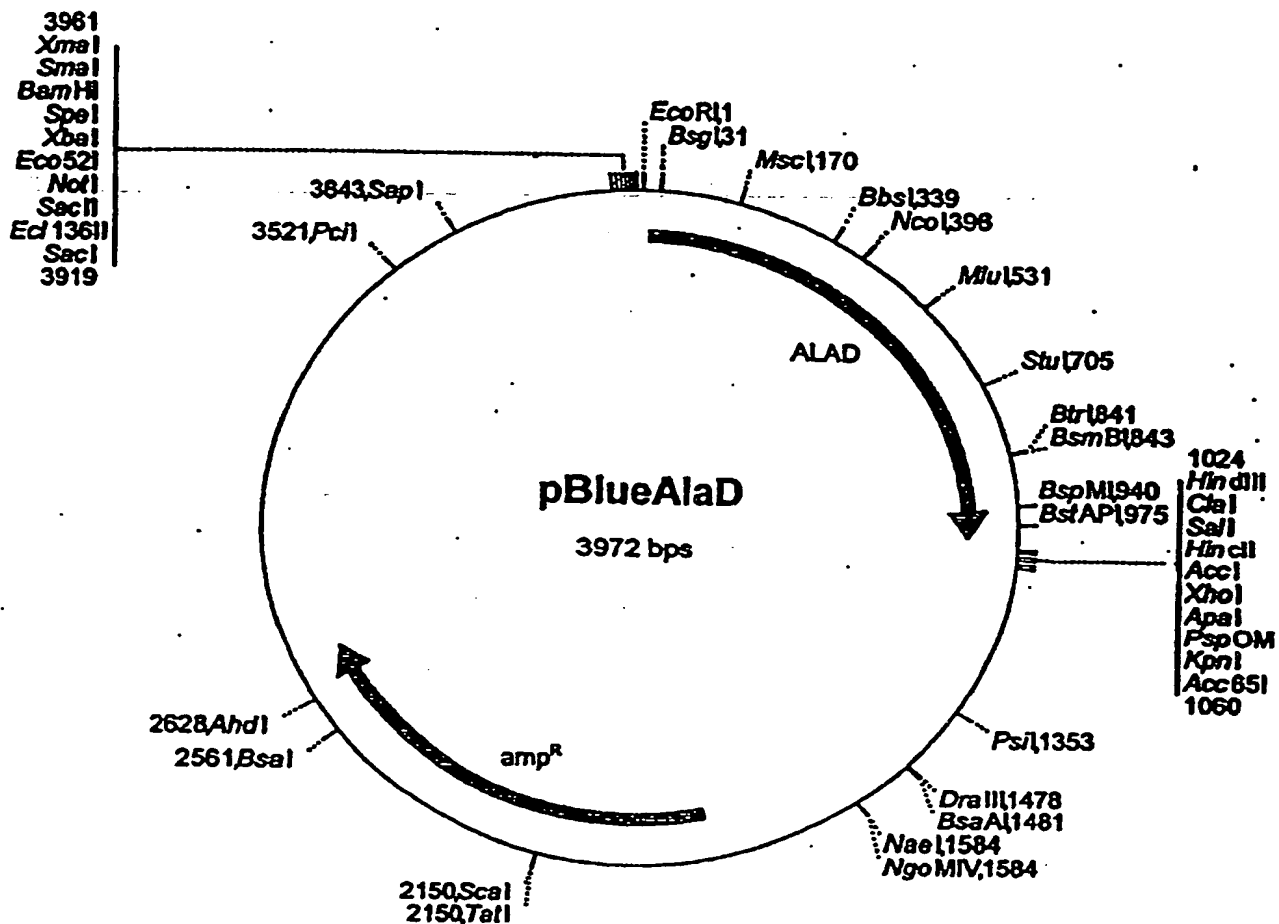


Fig. 38 37C

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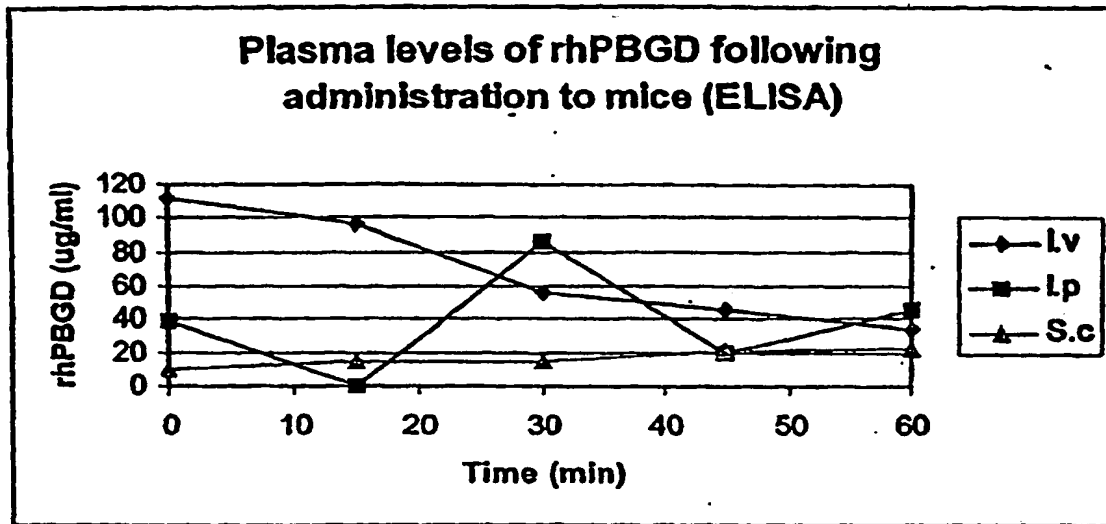


Figure 38: Plasma levels of rhPBGD following administration to mice.

~~Fig. 39~~

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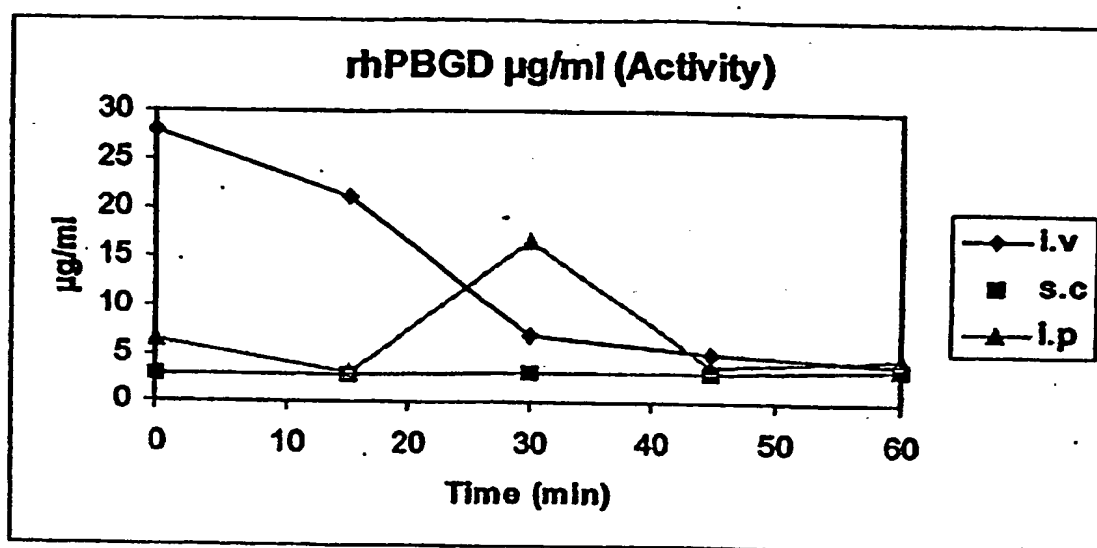


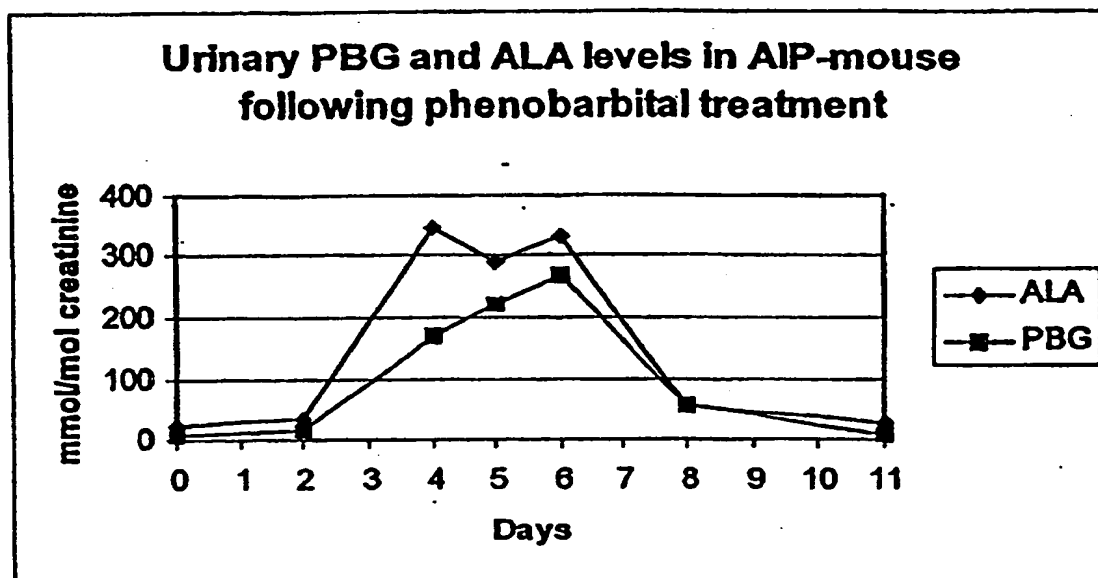
Figure 39: PBGD enzymatic activity in plasma following rhPBGD administration to mice

~~Fig. 40~~

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**Figure 40: Urinary content of PBG and ALA in AIP-mouse treated with phenobarbital.**

~~Fig. 41~~

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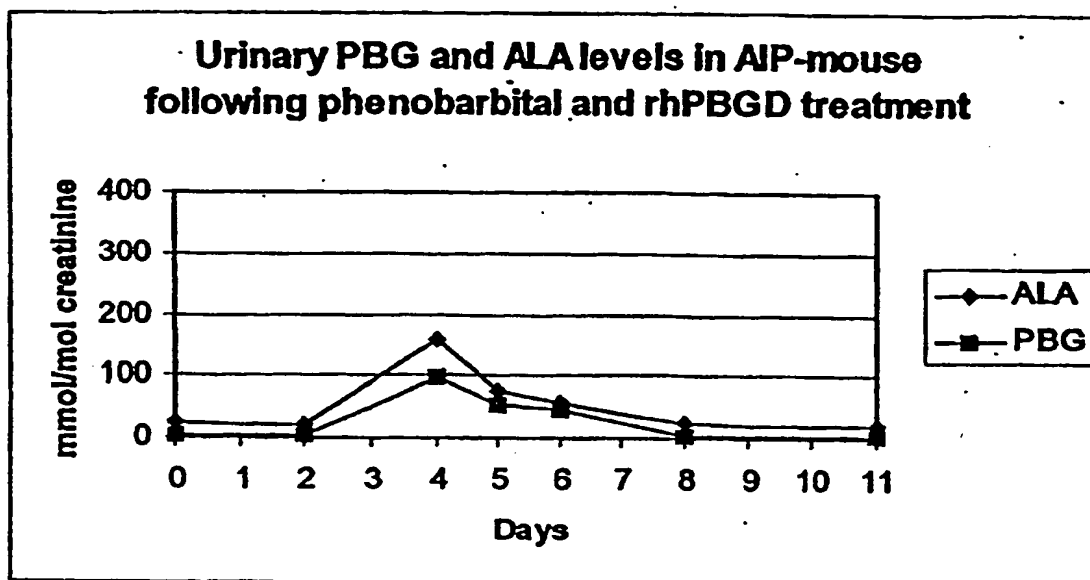


Figure 41: Urinary content of PBG and ALA in AIP-mouse treated with phenobarbital and rhPBGD. Mice were treated with an increasing dose of phenobarbital for 4 days (day 0-4,

~~Fig. 42~~

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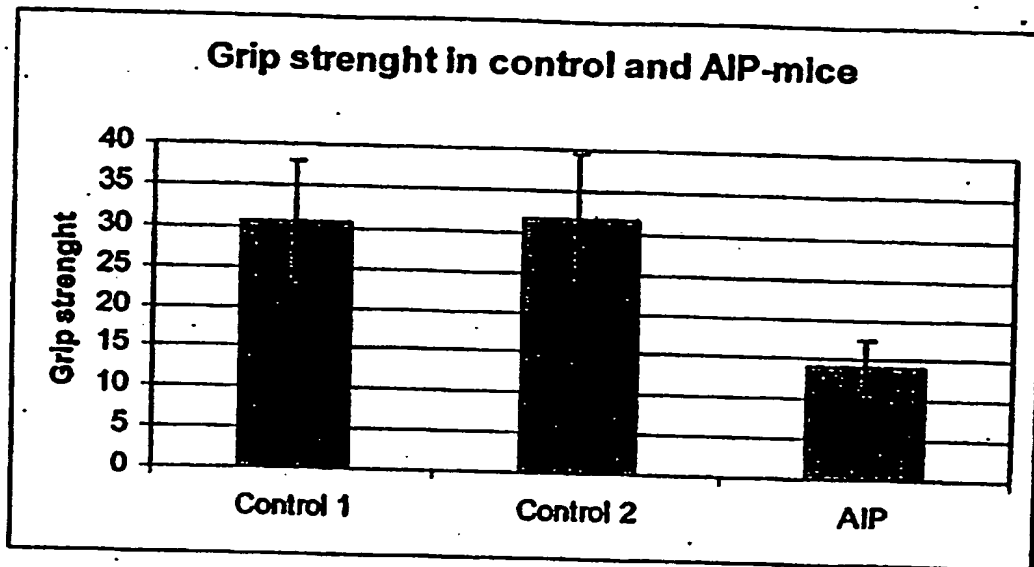


Figure 42: Grip strength analysis in control and AIP-mice. Grip strength were determined using a grip strength meter (Ugo Basile) in heterozygous control animals (control 1,  $n=5$ ), in wild type controls (control 2,  $n=5$ ) and in AIP-transgenic mice (AIP,  $n=5$ ).

~~Fig. 43~~

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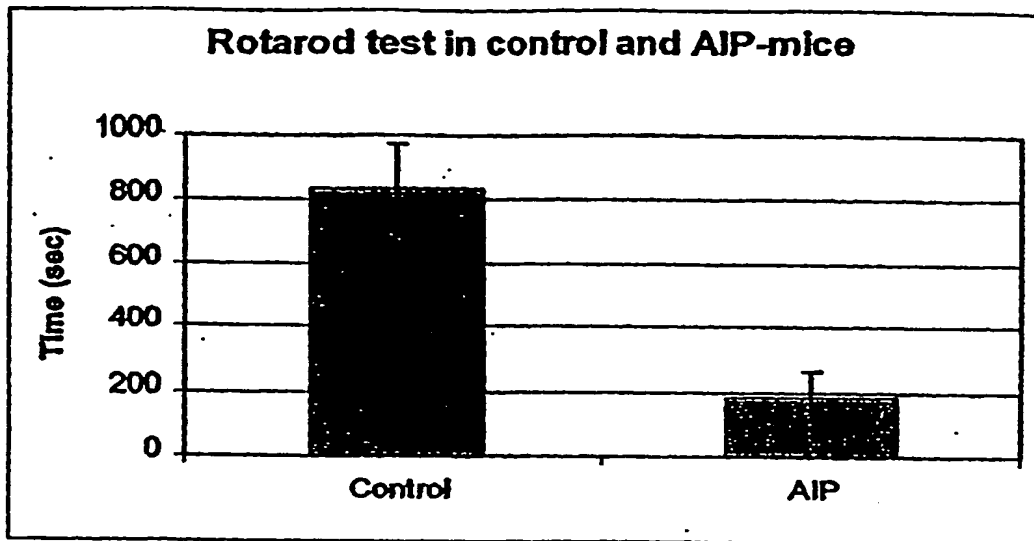


Figure 4 Rotarod analysis in control and AIP-mice. The rotarod analysis were determined using a rotarod treadmill (Ugo Basile) in wild type controls (control, n=5) and in AIP-transgenic mice (AIP, n=7).

Fig. ~~44~~ 43

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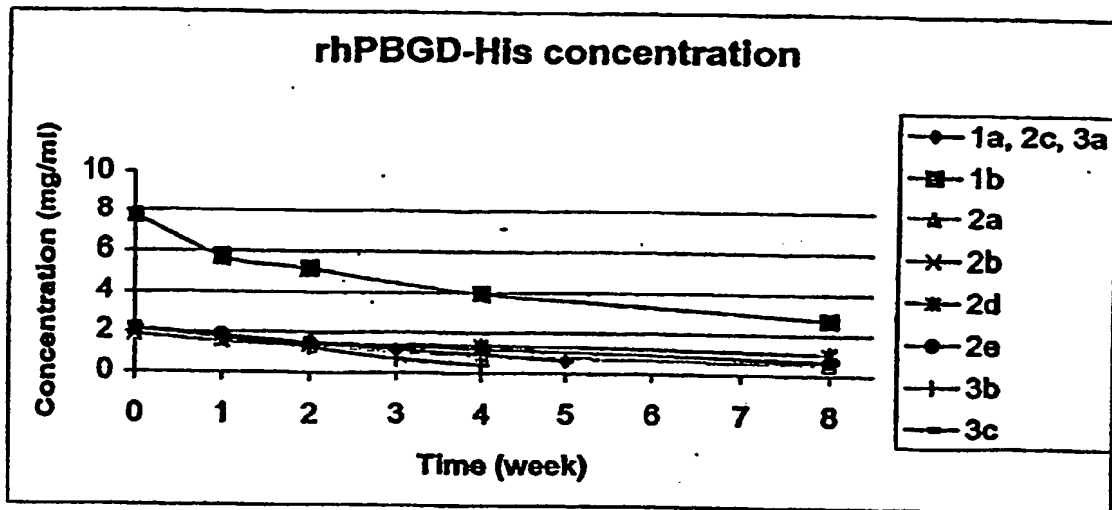


Figure 44. Enzyme concentration over 8 weeks at 40°C measured by HPLC. A decrease from 2 mg/ml to 0,5 mg/ml and 8 mg/ml to 2,5 was detected.

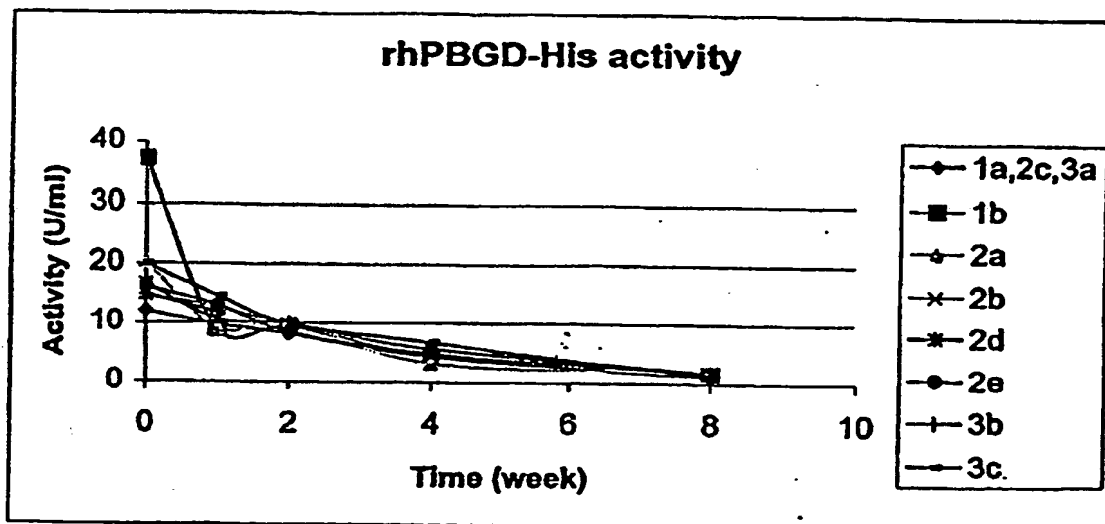


Figure 45. The enzyme activity measured over 8 weeks at 40°C. A significant decrease over the first week was seen for the high concentration sample, 1b. After two weeks the decrease rate was the same for all samples.

~~Fig. 45~~

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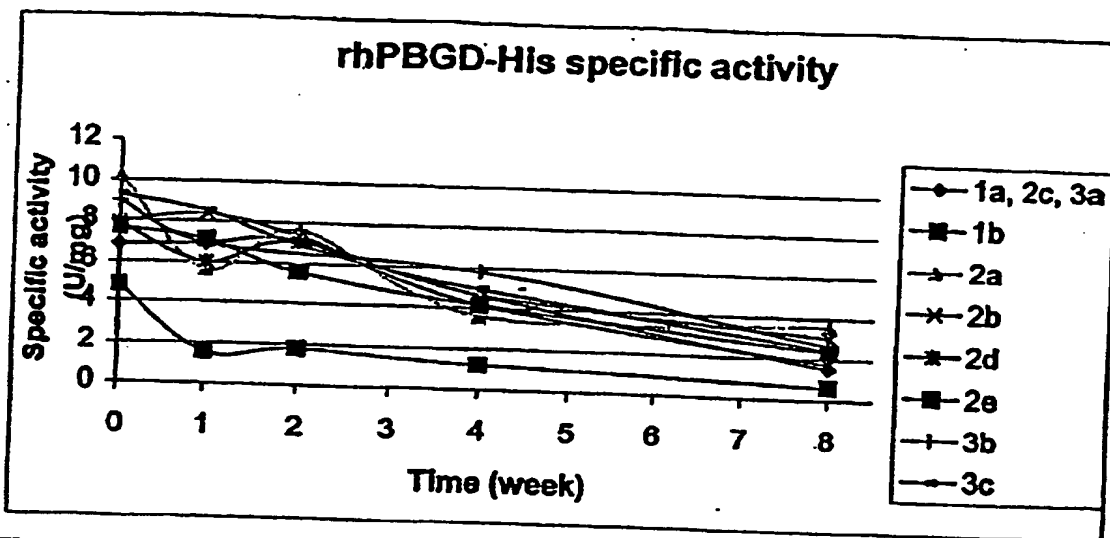


Figure 46. Enzyme specific activity measured during 8 weeks at 40°C. The activity was measured using the enzyme activity assay and the protein concentration was measured using HPLC.

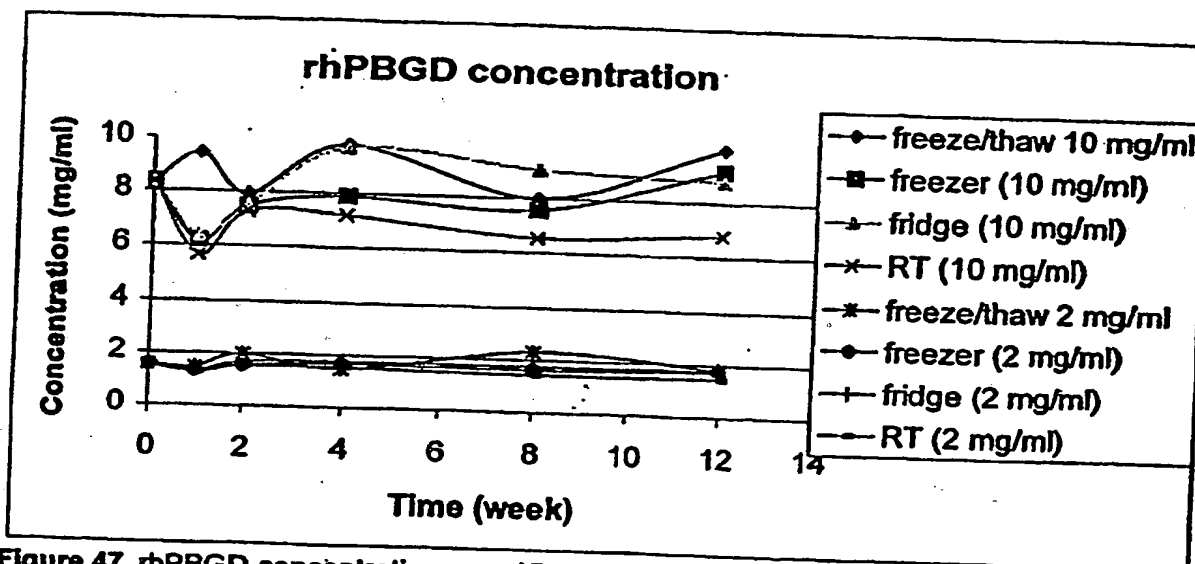


Figure 47. rhPBGD concentration over 12 weeks at -20°C (freezer), 5°C (fridge), 25°C (RT) and freeze/thawed at each sampling. The measurement was performed using HPLC.

~~Fig. 46~~

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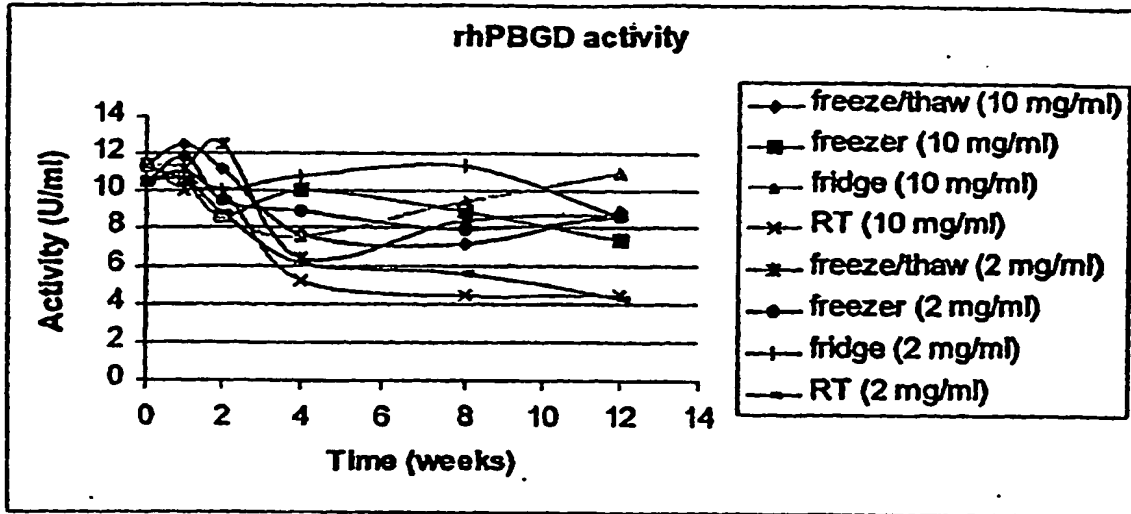


Figure 48. rhPBGD activity over 12 weeks at  $-20^{\circ}\text{C}$  (freezer),  $5^{\circ}\text{C}$  (fridge),  $25^{\circ}\text{C}$  (RT) and freeze/thawed at each sampling.

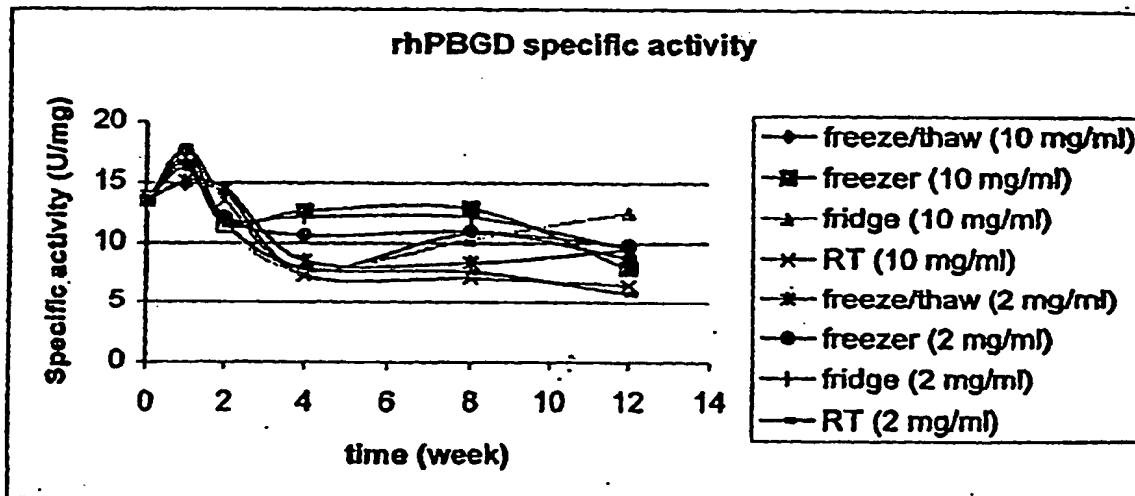


Figure 49. rhPBGD specific activity over 12 weeks at  $-20^{\circ}\text{C}$  (freezer),  $5^{\circ}\text{C}$  (fridge),  $25^{\circ}\text{C}$  (RT) and freeze/thawed at each sampling. Measurements were performed using enzyme activity assay and HPLC.

~~Fig. 47~~

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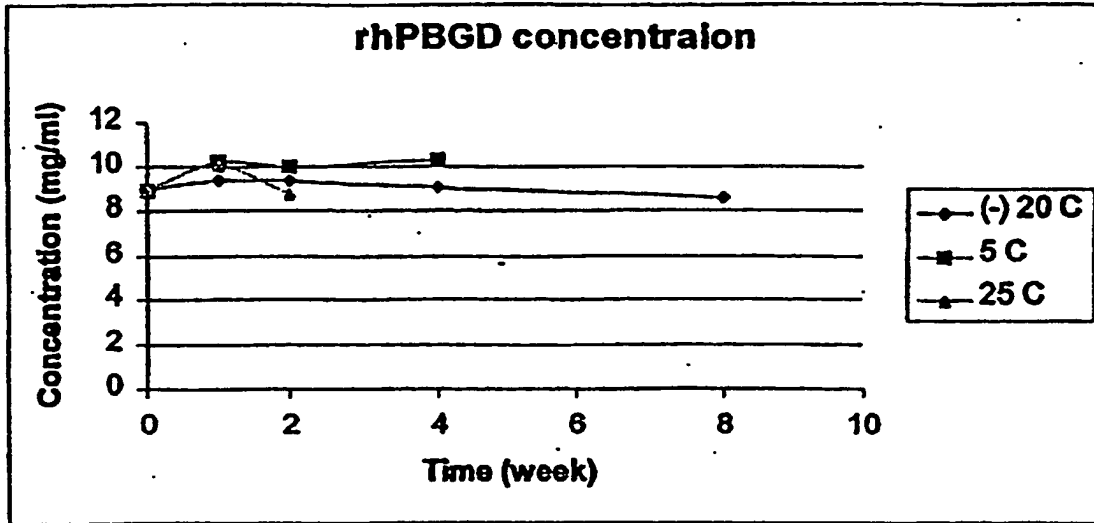


Figure 50. rhPBGD concentration measured over 8 weeks using BCA.

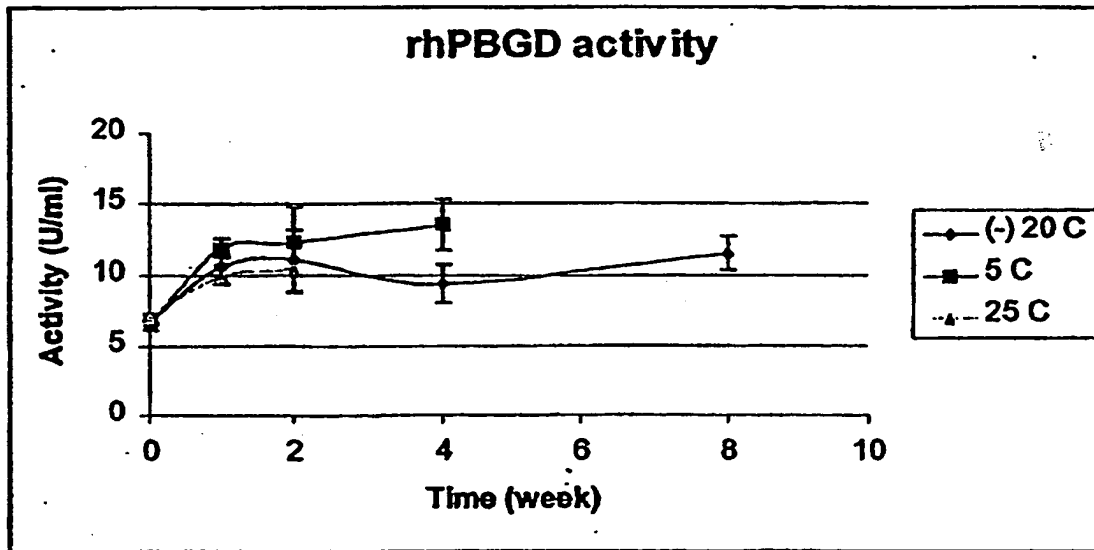


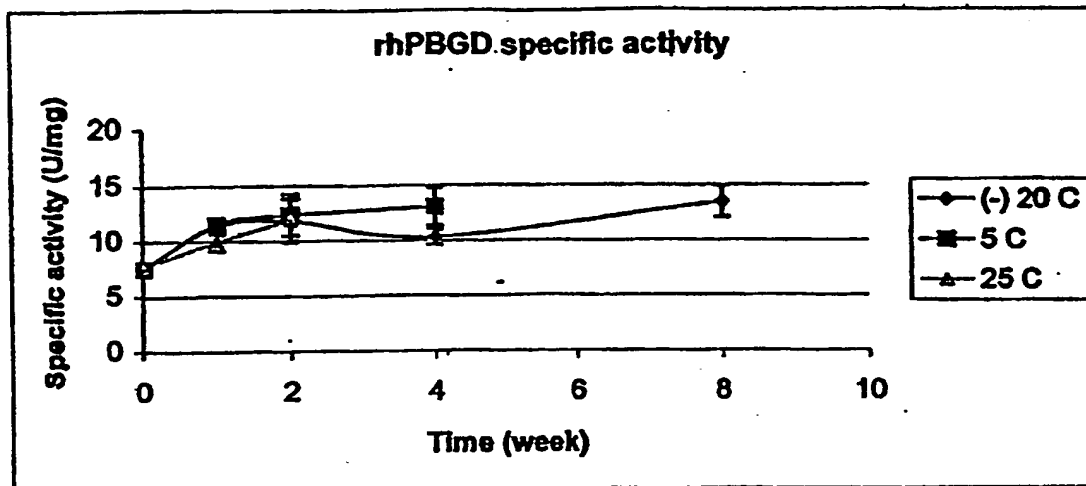
Figure 51. The rhPBGD activity measured over 8 weeks. The stability study has been performed under nitrogen at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ ,  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$  and at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

~~Fig. 48~~

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**Figure 52.** The specific rhPBGD activity measured using the enzyme activity assay and BCA protein concentration assay. The stability study has been performed under nitrogen at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ ,  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$  and at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

~~Fig. 49~~

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